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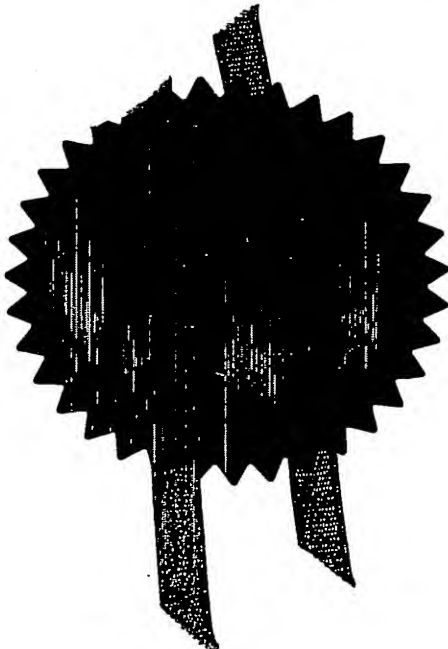
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### 1. Your reference

A2793-GB

### 2. Patent application number

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22 APR 2004

0408955.3

### 3. Full name, address and postcode of the or of each applicant (underline all surnames)

08711871001

Patents ADP number (if you know it)

4 AZA Bioscience nv  
Kapucijnenvoer 33  
B-3000 Leuven  
Belgium

If the applicant is a corporate body, give the country/state of its incorporation

BE

### 4. Title of the invention

Pteridine derivatives useful as pharmaceutical ingredients

### 5. Name of your agent (if you have one)

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postmark)

William E. Bird of Bird Goën & Co.  
Bird Goën & Co  
155 Regents Park Road  
London NW1 8BB  
United Kingdom

Patents ADP number (if you know it)

08675910001

### 6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number  
(if you know it)

Date of filing  
(day / month / year)

### 7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing  
(day / month / year)

### 8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if

YES

- a) any applicant named in part 3 is not an inventor, or
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**Patents Form 1/77**

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**Continuation sheets of this form**

Description	124 pages
Claim(s)	5 pages
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**Priority documents**

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Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77) 1

Request for substantive examination (Patents Form 10/77)

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11.

I/We request the grant of a patent on the basis of this application

Signature *William E. Bird* Date

William E. Bird 21 April 2004

12. Name and daytime telephone number of person to contact in the United Kingdom

0871 900 8130

Paulette Wilson

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## PTERIDINE DERIVATIVES USEFUL AS PHARMACEUTICAL INGREDIENTS

FIELD OF THE INVENTION

The invention relates to a class of novel pteridines. The invention further  
5 relates to pharmaceutical compositions including a broad class of pteridines  
especially for the prevention and/or the treatment of pathologic conditions  
such as, but not limited to, immune and auto-immune disorders, organ and  
cells transplant rejections, cell proliferative disorders, cardiovascular  
disorders, allergic conditions, disorders of the central nervous system and viral  
10 diseases.

The invention further relates to combined pharmaceutical preparations  
comprising one or more such pteridines and one or more known  
immunosuppressant drugs or antineoplastic drugs or anti-viral drugs.

This invention also relates to a method for the prevention and/or  
15 treatment of pathologic conditions such as, but not limited to, immune and  
autoimmune disorders, organ and cells transplant rejections, cell proliferative  
disorders, cardiovascular disorders, disorders of the central nervous system,  
allergic conditions and viral diseases by the administration of an effective  
amount of a pteridine derivative optionally combined with one or more known  
20 immunosuppressant drugs or antineoplastic drugs or anti-viral drugs.

The present invention also relates to the treatment of side effects of  
various chemotherapeutic drugs and/or of irradiation in cancer therapy. The  
present invention also relates to the treatment of septic shock, as well as toxic  
side effects, disorders and diseases related to or resulting from the exposure  
25 of patients to abnormally high levels of tumor necrosis factor-alpha  
(hereinafter referred as TNF- $\alpha$ ) in general, and particularly following the  
administration of TNF- $\alpha$  in cancer treatment in humans. This invention also  
relates to the treatment of radiotherapy-induced or chemotherapy-induced  
disorders such as mucositis, secondary myelodysplastic syndromes and  
30 radiation-induced graft-versus-host disease, and for the prevention and/or the  
treatment of injuries in cancer patients such as, but not limited to, apoptosis,  
radiation necrosis and nephrotoxicity following the administration of certain



chemotherapeutic drugs such as cisplatin in cancer treatment. Additionally the invention relates to the treatment of cachexia.

### BACKGROUND OF THE INVENTION

5           Several 2,4-diaminopteridine derivatives being substituted in the 6-position and/or the 7-position of the pteridine ring (according to standard atom numbering for said ring) are known in the art, e.g. from various sources of literature including Swiss Patent No. 231,852; British Patent No. 763,044; United States Patents No. 2,512,572; No. 2,581,889; No. 2,665,275; No. 2,667,486; No. 10 2,940,972; No. 3,081,230 and No. 5,047,405. Some of these substituted 2,4-diaminopteridine derivatives were disclosed in relationship with various medical uses, such as bacterial growth inhibitors, antineoplastic agents, anti-schistosomiasis activity, coronary dilating activity, diuretic and hypotensive activity, and anti-amnesic activity. In particular, U.S. Patent No. 2,940,972, 15 British Patent No. 2,143,232, EP-A-185,259 and EP-A-362,645 disclose very specific 2,4-diaminopteridine derivatives being substituted by piperidinyl, morpholinyl or pyrrolidinyl in the 7-position of the pteridine ring, some of them being useful for metastasis inhibition or treatment of tumor growth. Furthermore, Merz et al. in *J. Med. Chem.* (1998) 41:4733-4743 disclose 2-*N*- 20 acetyl piperazino-4-pyrrolidino-6-chloro-7-benzylamino and its use for inhibiting cAMP phosphodiesterase and malignant tumor cell growth.

          Nevertheless, there still is a continuous need in the art for highly active and highly specific therapeutic compounds, such as, but not limited to, drugs for treating immune and autoimmune disorders, organ and cells transplant 25 rejections, cell proliferative disorders, cardiovascular disorders, disorders of the central nervous system, allergic conditions and viral diseases. In particular, there is a strong need in the art to provide immunosuppressive compounds, antineoplastic drugs and anti-viral drugs which are active in a minor dose and/or at a specific locus in order to replace existing drugs having 30 significant side effects and to decrease treatment costs.

          Currently used immunosuppressive drugs include antiproliferative agents, such as methotrexate (a 2,4-diaminopteridine derivative disclosed by U.S. Patent No. 2,512,572), azathioprine, and cyclophosphamide. Since these

drugs affect mitosis and cell division, they have severe toxic effects on normal cells with high turn-over rate such as bone marrow cells and the gastrointestinal tract lining. Accordingly, marrow depression and liver damage are common side effects of these antiproliferative drugs.

5        Anti-inflammatory compounds used to induce immunosuppression include adrenocortical steroids such as dexamethasone and prednisolone. The common side effects observed with the use of these compounds are frequent infections, abnormal metabolism, hypertension, and diabetes.

10        Other immunosuppressive compounds currently used to inhibit lymphocyte activation and subsequent proliferation include cyclosporine, tacrolimus and rapamycin. Cyclosporine and its relatives are among the most commonly used immunosuppressant drugs. Cyclosporine is typically used for preventing or treating organ rejection in kidney, liver, heart, pancreas, bone marrow, and heart-lung transplants, as well as for the treatment of  
15        autoimmune and inflammatory diseases such as Crohn's disease, aplastic anemia, multiple-sclerosis, myasthenia gravis, uveitis, biliary cirrhosis, etc. However, cyclosporines suffer from a small therapeutic dose window and severe toxic effects including nephrotoxicity, hepatotoxicity, hypertension, hirsutism, cancer, and neurotoxicity.

20        Additionally, monoclonal antibodies with immunosuppressant properties, such as OKT3, have been used to prevent and/or treat graft rejection. Introduction of such monoclonal antibodies into a patient, as with many biological materials, induces several side-effects, such as dyspnea. Within the context of many life-threatening diseases, organ transplantation is  
25        considered a standard treatment and, in many cases, the only alternative to death. The immune response to foreign cell surface antigens on the graft, encoded by the major histo-compatibility complex (hereinafter referred as MHC) and present on all cells, generally precludes successful transplantation of tissues and organs unless the transplant tissues come from a compatible  
30        donor and the normal immune response is suppressed. Other than identical twins, the best compatibility and thus, long term rates of engraftment, are achieved using MHC identical sibling donors or MHC identical unrelated cadaver donors. However, such ideal matches are difficult to achieve. Further,

with the increasing need of donor organs an increasing shortage of transplanted organs currently exists. Accordingly, xenotransplantation has emerged as an area of intensive study, but faces many hurdles with regard to rejection within the recipient organism.

- 5           The host response to an organ allograft involves a complex series of cellular interactions among T and B lymphocytes as well as macrophages or dendritic cells that recognize and are activated by foreign antigen. Co-stimulatory factors, primarily cytokines, and specific cell-cell interactions, provided by activated accessory cells such as macrophages or dendritic cells
- 10 are essential for T-cell proliferation. These macrophages and dendritic cells either directly adhere to T-cells through specific adhesion proteins or secrete cytokines that stimulate T-cells, such as IL-12 and IL-15. Accessory cell-derived co-stimulatory signals stimulate activation of interleukin-2 (IL-2) gene transcription and expression of high affinity IL-2 receptors in T-cells. IL-2 is
- 15 secreted by T lymphocytes upon antigen stimulation and is required for normal immune responsiveness. IL-2 stimulates lymphoid cells to proliferate and differentiate by binding to IL-2 specific cell surface receptors (IL-2R). IL-2 also initiates helper T-cell activation of cytotoxic T-cells and stimulates secretion of interferon- $\gamma$  which in turn activates cytotoxic properties of macrophages.
- 20 Furthermore, IFN- $\gamma$  and IL-4 are also important activators of MHC class II expression in the transplanted organ, thereby further expanding the rejection cascade by enhancing the immunogenicity of the grafted organ. The current model of a T-cell mediated response suggests that T-cells are primed in the T-cell zone of secondary lymphoid organs, primarily by dendritic cells. The initial
- 25 interaction requires cell to cell contact between antigen-loaded MHC molecules on antigen-presenting cells (hereinafter referred as APC) and the T-cell receptor/CD3 complex on T-cells. Engagement of the TCR/CD3 complex induces CD154 expression predominantly on CD4 T-cells that in turn activate the APC through CD40 engagement, leading to improved antigen
- 30 presentation. This is caused partly by upregulation of CD80 and CD86 expression on the APC, both of which are ligands for the important CD28 co-stimulatory molecule on T-cells. However, engagement of CD40 also leads to prolonged surface expression of MHC-antigen complexes, expression of

ligands for 4-1BB and OX-40 (potent co-stimulatory molecules expressed on activated T-cells). Furthermore, CD40 engagement leads to secretion of various cytokines (e.g., IL-12, IL-15, TNF- $\alpha$ , IL-1, IL-6, and IL-8) and chemokines, all of which have important effects on both APC and T-cell activation and maturation. Similar mechanisms are involved in the development of auto-immune disease, such as type I diabetes. In humans and non-obese diabetic mice, insulin-dependent diabetes mellitus results from a spontaneous T-cell dependent auto-immune destruction of insulin-producing pancreatic  $\beta$  cells that intensifies with age. The process is preceded by infiltration of the islets with mononuclear cells (insulitis), primarily composed of T lymphocytes. A delicate balance between auto-aggressive T-cells and suppressor-type immune phenomena determines whether expression of auto-immunity is limited to insulitis or not. Therapeutic strategies that target T-cells have been successful in preventing further progress of the auto-immune disease. These include neonatal thymectomy, administration of cyclosporine, and infusion of anti-pan T-cell, anti-CD4, or anti-CD25 (IL-2R) monoclonal antibodies. The aim of all rejection prevention and auto-immunity reversal strategies is to suppress the patient's immune reactivity to the antigenic tissue or agent, with a minimum of morbidity and mortality. Accordingly, a number of drugs are currently being used or investigated for their immunosuppressive properties. As discussed above, the most commonly used immunosuppressant is cyclosporine, which however has numerous side effects. Accordingly, in view of the relatively few choices for agents effective at immunosuppression with low toxicity profiles and manageable side effects, there exists a need in the art for identification of alternative immunosuppressive agents and for agents acting as complement to calcineurin inhibition.

The metastasis of cancer cells represents the primary source of clinical morbidity and mortality in the large majority of solid tumors. Metastasis of cancer cells may result from the entry of tumor cells into either lymphatic or blood vessels. Invasion of lymphatic vessels results in metastasis to regional draining lymph nodes. From the lymph nodes, melanoma cells for example tend to metastasize to the lung, liver, and brain. For several solid tumors,

including melanoma, the absence or the presence of lymph nodes metastasis is the best predictor of patient survival. Presently, to our knowledge, no treatment is capable of preventing or significantly reducing metastasis. Hence, there is a need in the art for compounds having such anti-metastasis effect for a suitable treatment of cancer patients.

In the field of allergy, IgE is well known for inducing allergy mainly by stimulating mast cells to release histamine. Also, asthma, being characterized by inflammation of airway and bronchospasm, is mainly induced by Th2 cytokines such as IL-5, IL-10 or IL-13. Therefore there is a need in the art for compounds that efficiently inhibit the release of these Th2 cytokines.

There is also a need in the art to improve therapeutic efficiency by providing pharmaceutical compositions or combined preparations exhibiting a synergistic effect as a result of combining two or more immunosuppressant drugs, or antineoplastic drugs or anti-viral drugs or anti-histamine drugs.

Septic shock is a major cause of death in intensive care units (about 150,000 estimated deaths annually in the United States of America, despite treatment with intravenous antibiotics and supportive care) for which very little effective treatment is available at present. Patients with severe sepsis often experience failures of various systems in the body, including the circulatory system, as well as kidney failure, bleeding and clotting. Lipopolysaccharide (hereinafter referred as LPS) is the primary mediator of Gram-negative sepsis, the most common form of sepsis, by inducing the production of a whole array of macrophage-derived cytokines (such as TNF- $\alpha$ ; interleukins such as IL-1, IL-6, IL-12; interferon-gamma (hereinafter referred IFN- $\gamma$ ), etc.). These cytokines may induce other cells (e.g. T cells, NK cells) to make cytokines as well (e.g. IFN- $\gamma$ ). In addition, other macrophage products (e.g. nitric oxide, hereinafter referred as NO) may also play a role in the pathogenesis of toxic shock. These substances (e.g. NO) may be induced directly due to microbial interactions or indirectly through the action of proinflammatory cytokines. LPS binds to a serum protein known as LPB and the LPS-LPB complex thus formed is recognized by the CD14 toll-like receptor 4 (hereinafter referred as Tlr 4) complex on mononuclear phagocytes. Tlr4 is a signal transducing unit, the activation of which results in the release of

mediators such as  $\text{TNF-}\alpha$ ,  $\text{IL-1}\alpha$ ,  $\text{IL-1}\beta$  and  $\text{IL-6}$ . These cytokines are important for the pathogenesis of shock. Their administration produces the clinical symptoms of septic shock and their blockade partially protects against LPS-induced lethal shock.

5        Current therapeutic strategies for the treatment of septic shock are directed against LPS (e.g. antibodies against LPS or LBP-34-23) or against the cytokines induced by LPS (e.g. TNF antibodies) or against the receptor for LPS (e.g. CD14). Unfortunately the initial clinical data of these approaches are very disappointing and illustrate the redundancy of receptors and mediators  
10 involved in the pathogenesis of toxic shock. For instance flagellin seems to be another toxin that plays a role in Gram-negative Salmonella shock syndrome and that cannot be prevented or treated by therapeutic strategies directed specifically at LPS.

      Clinical trials in humans with  $\text{TNF-}\alpha$  blocking antibodies (such as the IL-  
15 1 receptor antagonist or PAF receptor antagonists) have been unsuccessful yet, as have been approaches to down regulate inflammation (e.g. using prednisolone) or to block endotoxins. These products must be administered very early after the onset of the disease, which is in most cases not possible.

      The only drug currently approved by health authorities for the treatment  
20 of adult patients with the most serious forms of sepsis, including septic shock, is a genetically engineered version of a naturally occurring human protein, Activated Protein C, known as Xigris® or drotrecogin-alpha which shows only moderate efficacy. Furthermore, because Activated Protein C interferes with blood clotting, the most serious side effect associated with Xigris® is bleeding,  
25 including bleeding that causes stroke. Thus Xigris® is contra-indicated for patients who have active internal bleeding, or who are more likely to bleed because of certain medical conditions including recent strokes, recent head or spinal surgery or severe head trauma. Because treatment with Xigris® comes with potentially serious risks, the benefits and risks of treatment with Xigris®  
30 must be carefully weighed for each individual patient.

      Therefore there is a strong need in the art for new medications, either alone or in combination with the currently suggested treatments, for treating

the most serious forms of life-threatening illnesses caused by severe infection, such as septic shock.

TNF- $\alpha$  is generally considered to be the key mediator in the mammalian response to bacterial infection. It is a strong pro-inflammatory agent that will affect the function of almost any organ system, either directly or by inducing the formation of other cytokines like IL-1 or prostaglandines. TNF- $\alpha$  is also a potent anti-tumor agent. If administered in small quantities to humans, it causes fever, headache, anorexia, myalgia, hypotension, capillary leak syndrome, increased rates of lipolysis and skeletal muscle protein degradation (including cachexia). Its use in cancer treatment is therefore very much limited by its severe side effects.

TNF- $\alpha$ , a pleiotropic cytokine produced mainly by activated macrophages, exerts an *in vitro* cytotoxic action against transformed cells and *in vivo* anti-tumor activities in animal models. However, despite the fact that TNF- $\alpha$  is used in cancer patients especially to treat melanoma and sarcoma, the major problem hampering its use is toxicity. Indeed, TNF- $\alpha$  induces shock-like symptoms such as bowel swelling and damage, liver cell necrosis, enhanced release of inflammatory cytokines such as IL-1 or IL-6, and hypotension probably due to the release of inducers of vessels dilatation such nitric oxide and other proinflammatory cytokines. Cardiovascular toxicity is usually dose-limiting. Hypotension can be severe with systolic blood pressure below 60 mm Hg. Respiratory compromise is common after treatment with TNF- $\alpha$  and may require mechanical ventilation. Upper as well as lower digestive tract symptoms are also common in this type of treatment. Nausea and vomiting can be distressing and in some cases dose-limiting. Watery diarrhea is frequently observed. Neurological sequelae of treatment with TNF- $\alpha$  can also occur.

Hence, compounds that inhibit the toxic effects of TNF- $\alpha$  but that do not inhibit TNF- $\alpha$  anti-tumor effect are highly desirable for the treatment of cancer patients. Presently, several clinical trials involving TNF- $\alpha$  are being developed for the cancer of organs such as liver, lung, kidney and pancreas, which are based on a procedure including the steps of organ isolation, injection of TNF- $\alpha$  into the isolated organ, and reperfusion of the treated organ. However, even

for isolated organ perfusion. some TNF- $\alpha$  usually escapes to the general blood circulation and leads to the mortality of about 10% of the patients thus treated. Many patients treated by this procedure also require intensive care unit rescue to cope with the toxic side-effects of such TNF- $\alpha$  treatment.

5 Combined treatment of TNF- $\alpha$  with alkylating drugs in an isolated organ perfusion model has received considerable attention. TNF- $\alpha$  is currently successfully used in isolated limb perfusion of human cancer patients and, in combination with melphalan and interferon-gamma, against melanoma, sarcomas and carcinomas.

10 The gastrointestinal mucosa is very sensitive to chemotherapeutic drugs. Mucositis caused by chemotherapy usually begins rapidly after initiation of the treatment with inflammation and ulceration of the gastrointestinal tract and leading to diarrhea. Severe, potentially life-threatening, diarrhea may require interruption of the chemotherapeutic treatment and subsequent dose reduction of the therapeutic agent. The oral cavity is often the place of severe side effects from cancer therapy that adversely affects the quality of life of the patient and its ability to tolerate the therapy. These side effects can be caused by radiotherapy as well as chemotherapy. A relationship between both serum and mucosal levels of  
15 TNF- $\alpha$  and IL-1 correlates with nonhematologic toxicities, including mucositis.  
20

Radiation injuries occurring e.g. after a single high-dose irradiation include apoptosis as well as radiation necrosis. Even normal tissues protected by shielding during irradiation may be considerably damaged. It was found in experimental animal models that the radiation injuries after a single high-dose  
25 irradiation typically used for the treatment of various malignant tumors consist of radiation necrosis and apoptosis, which were correlated with the expression of TNF- $\alpha$  and TGF- $\beta$ 1.

Irradiation may induce graft-versus-host disease (hereinafter referred as GVHD) in cancer patients. This disease may occur especially in patients  
30 receiving allogeneic bone marrow transplantation as a treatment for cancers such as leukemia or lymphoma and can lead to the death of about 25% of the relevant patients. Before bone marrow transplantation, leukaemia patients for example receive either total body or total lymphoid irradiation to suppress their



immune system. However, such irradiation induces not only necrosis but also the release of proinflammatory cytokines mainly TNF- $\alpha$ , IL-1 and IL-6 which in turn induce direct host tissues inflammation and activation of donor cells against host antigens leading to GVHD.

- 5 Cisplatin is an effective chemotherapeutic agent used in the treatment of a wide variety of both pediatric and adult malignancies, including testicular, germ cell, head and neck (cervical), bladder and lung cancer. Dose-dependent and cumulative nephrotoxicity is the major side effect of cisplatin, sometimes requiring a reduction in dose or discontinuation of the treatment.
- 10 Other side effects of cisplatin include kidney damage, loss of fertility, harmful effect on a developing baby, temporary drop in bone marrow function causing drop in white blood cell count, anaemia, drop in platelets causing bleeding, loss of appetite, numbness or tingling in limbs, loss of taste, allergic reactions, and hearing disorders (difficulty in hearing some high-pitched sounds,
- 15 experiencing ringing in the ears). Blurred vision may also be a side effect with high doses of cisplatin. It was shown that TNF- $\alpha$  is a key element in a network of proinflammatory chemokines and cytokines activated in the kidney by cisplatin. Blockade of TNF- $\alpha$  action would prevent the activation of this cytokine network and would provide protection against cisplatin nephrotoxicity.
- 20 Hence, compounds that inhibit the toxic effects of cisplatin but that do not inhibit cisplatin anti-tumor effects are highly desirable for the treatment of cancer patients.

- A surplus of TNF- $\alpha$  also causes a dramatic change of endothelial cells. In particular, TNF- $\alpha$  is an important mediator of skeletal muscle degeneration
- 25 associated with cachexia, a debilitating syndrome characterized by extreme weight loss and whole-body wasting. Cachexia is usually a secondary condition whereby there is excessive tissue catabolism in combination with deficient anabolism. It is frequently seen in patients afflicted with chronic diseases such as cancer, cardiopulmonary diseases, aging, malabsorptive disorders, excessive physical stress, eating disorders and acquired
- 30 immuno-deficiency syndrome (AIDS). Some authors consider that the elevated TNF- $\alpha$  values found in at least 50% of cancer patients in the active stage of the disease can result in cachexia. TNF- $\alpha$  levels in clinically healthy

adults, as well as in adult cancer patients, are well documented. for instance by Nenova et al. in *Archives of Hellenic Medicine* (2000) 17:619-621. Serum TNF- $\alpha$  concentrations in healthy children as well as in children with malignancies are documented for instance by Saarinen et al. in *Cancer Research* (1990) 50:592-595. A very significant proportion of cancer mortalities result from cachexia rather than from tumor burden. Chronic wasting disease (cachexia) may result when excessive cellular damage results in the release of substances (TNF- $\alpha$ , collagenase, hyaluronidase) that further catabolize the so-called healthy tissue resulting in an inability to assimilate nutrients required for anabolic restructuring of associated tissue.

Infants infected with human immunodeficiency virus type 1 (HIV-1) show growth retardation and severe weight loss that can lead to death. The overproduction of certain cytokines has been implicated as a possible cause for this. For instance, according to Rautonen et al. in *AIDS* (1991) 5:1319-1325, serum IL-6 concentrations are elevated and associated with elevated TNF- $\alpha$  concentrations in children with HIV infection. Swapan et al. in *Journal of Virology* (2002) 76:11710-11714 have shown that reduction of TNF- $\alpha$  levels by either anti-TNF- $\alpha$  antibodies or human chorionic gonadotropin inhibits the expression of HIV-1 proteins and prevents cachexia and death.

Very few drugs have been suggest at present for the treatment of cachexia. Some high-dose progestins like megestrol acetate, an agent used for the treatment of metastatic breast cancer, and medroxyprogesterone acetate were shown in randomized clinical trials to provide a statistically significant advantage as regards improved appetite and body weight gain. Hence, compounds that stimulate appetite and body weight gain without inhibiting the anti-tumor effect or anti-viral effect of co-administered drugs are highly desirable for the treatment of cachexia. More specifically, there is a need in the art for treating cachexia by the administration of compounds that reduce TNF- $\alpha$  levels in the serum of humans.

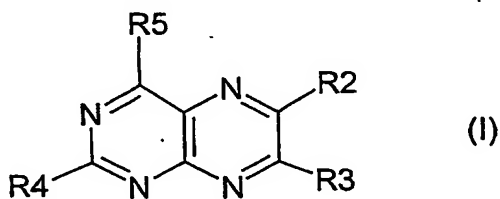
TNF- $\alpha$  is also suspected to play a role, through a possible dual action in the hematopoietic environment, in the development of hematologic malignancies such as idiopathic myelodysplastic syndromes occurring most

often in elderly people but also occasionally in children, these syndromes being currently regarded as the early phase of acute leukemia.

There is a strong need in the art to improve, or to provide alternatives to, the existing prophylactic or therapeutic solutions to all the aforesaid diseases. Meeting these various needs in the art constitutes the main goal of the present invention.

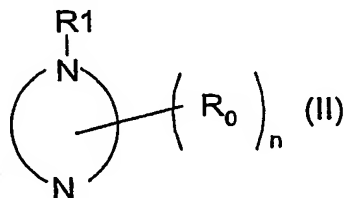
### SUMMARY OF THE INVENTION

In a first embodiment, the present invention relates to a group of novel pteridine derivatives having the general formula (I):

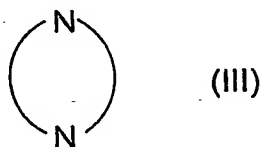


wherein:

- one or more of the substituents  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  of the pteridine ring is independently selected from groups represented by the general formula (II):



wherein:



15

schematically represents a saturated or partly unsaturated heterocyclic ring with at least two nitrogen atoms in the said heterocyclic ring and with a total of 5 to 7 atoms in the said heterocyclic ring, and optionally with one or more other heteroatoms (e.g. oxygen or sulfur) in the said heterocyclic ring or attached to one or more carbon atoms of said heterocyclic ring (for instance in the form of a carbonyl or thiocarbonyl group), wherein one of said at least two nitrogen

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atoms in the heterocyclic ring is attached to a carbon atom of the pteridine ring at any of positions 2, 4, 6 or 7 of the pteridine ring, wherein the said heterocyclic ring may be fused to one or more aromatic hydrocarbon rings, and wherein:

- 5       - each substituent  $R_0$  of the heterocyclic ring (III) is a group independently selected from the group consisting of halogen, nitro,  $C_{1-7}$  alkyl (optionally containing one or more functions or radicals selected from the group consisting of halogen, carbonyl, thiocarbonyl, hydroxyl, sulfhydryl,  $C_{1-7}$  alkoxy, thio  $C_{1-7}$  alkyl, thio  $C_{3-10}$  cycloalkyl, acetal, thio-acetal, imino, oximino, alkyloximino, amino-acid, cyano, (thio)carboxylic acid, (thio)carboxylic acid ester or amide, nitro, amino,  $C_{1-7}$  alkylamino, 10       cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, aryl-amino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl and sulfonamido),  $C_{3-7}$  alkenyl,  $C_{2-7}$  alkynyl, halo  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, 15       aryl, arylalkyl, alkylaryl, alkylacyl, arylacyl, hydroxyl, sulfhydryl; amino,  $C_{1-7}$  alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino,  $C_{1-7}$  alkoxy,  $C_{3-10}$  cycloalkoxy, aryloxy, arylalkyloxy, 20       oxyheterocyclic, heterocyclic-substituted alkyloxy, thio  $C_{1-7}$  alkyl, thio  $C_{3-10}$  cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, hydroxylamino, cyano, (thio)carboxylic acid or esters or thioesters or amides or thioamides thereof;
- $n$  is an integer from 0 to 6;
- 25       -  $R_1$  is a radical selected from the group consisting of formyl, acyl, thioacyl, amide, thioamide, sulfonyl, sulfinyl, carboxylate, thiocarboxylate, amino-substituted acyl, alkoxyalkyl,  $C_{3-10}$  cycloalkyl-alkyl,  $C_{3-10}$  cycloalkyl, dialkylaminoalkyl, heterocyclic-substituted alkyl, acyl-substituted alkyl, thioacyl-substituted alkyl, amido-substituted alkyl, thioamido-substituted alkyl, carboxylato-substituted alkyl, thiocarboxylato-substituted alkyl, (amino-substituted acyl)alkyl, heterocyclic, carboxylic acid ester,  $\omega$ -cyanoalkyl,  $\omega$ -carboxylic ester-alkyl, halo  $C_{1-7}$  alkyl,  $C_{2-7}$  30       alkenyl,  $C_{2-7}$  alkynyl, arylalkenyl, aryloxyalkyl, arylalkyl and aryl,

wherein the aryl moiety of each of said arylalkenyl, aryloxyalkyl, arylalkyl and aryl radicals is optionally substituted with one or more substituents independently selected from the group consisting of halogen, C<sub>1-7</sub> alkyl, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, halo C<sub>1-7</sub> alkyl, nitro, hydroxyl, sulfhydryl, amino, C<sub>1-7</sub> alkoxy, C<sub>3-10</sub> cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, thioaryl, thio-heterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thioureido, sulfon-amido, hydroxylamino, alkoxyamino, mercaptoamino, thioalkyl-amino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino, cycloalkylamino, alkenyl-amino, cycloalkenylamino, alkynylamino, arylamino, aryl-alkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; and

- wherein the remaining of the substituents R<sub>2</sub>, R<sub>3</sub>, R<sub>4</sub> and R<sub>5</sub> of the pteridine ring is independently selected from the group consisting of hydrogen; halogen; C<sub>1-7</sub> alkyl; C<sub>2-7</sub> alkenyl; C<sub>2-7</sub> alkynyl; halo C<sub>1-7</sub> alkyl; carboxy C<sub>1-7</sub> alkyl; carboxyaryl; C<sub>1-7</sub> alkoxy; C<sub>3-10</sub> cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; thio C<sub>1-7</sub> alkyl; thio C<sub>3-10</sub> cycloalkyl; thioaryl; thioheterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; mercapto-amino; acylamino; thio-acylamino; alkoxyamino; thioalkylamino; acetal; thio-acetal; carboxylic acid; carboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; thiocarboxylic acid; thiocarboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; hydroxyl; sulfhydryl; nitro; cyano; carbamoyl; thiocarbamoyl; ureido; thio-ureido; amino; alkylamino; cycloalkyl-amino; alkenylamino; cycloalkenylamino; alkynylamino; arylamino; arylalkyl-amino; hydroxyalkylamino; mercaptoalkylamino; heterocyclic amino; heterocyclic-substituted alkylamino; oximino; alkyloximino; hydrazino; alkylhydrazino; phenylhydrazino; cysteinyl acid, esters, thioesters, halides, anhydrides, amides and thioamides thereof; aryl optionally substituted with one or more

substituents selected from the group consisting of halogen, C<sub>1-7</sub> alkyl, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, halo C<sub>1-7</sub> alkyl, nitro, hydroxyl, sulfhydryl, amino, C<sub>1-7</sub> alkoxy, C<sub>3-10</sub> cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, thioaryl, thio-

5 heterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thio-ureido, sulfonamido, hydroxylamino, alkoxyamino, mercaptoamino, thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides

10 thereof, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; optionally substituted heterocyclic radicals; aromatic or heterocyclic substituents substituted with an aliphatic spacer between the pteridine ring

15 and the aromatic or heterocyclic substituent, whereby said aliphatic spacer is a branched or straight, saturated or unsaturated aliphatic chain of 1 to 4 carbon atoms which may contain one or more functions, atoms or radicals selected from the group consisting of carbonyl, thiocarbonyl, hydroxyl, thiol, ether, thioether, acetal, thioacetal, amino, imino, oximino, alkyloximino, amino-

20 acid, cyano, acylamino, thioacylamino, carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic acid or ester or thioester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino,

25 arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; branched or straight, saturated or unsaturated aliphatic chains of 1 to 7 carbon atoms optionally containing one or more functions selected from the group consisting of halogen, carbonyl,

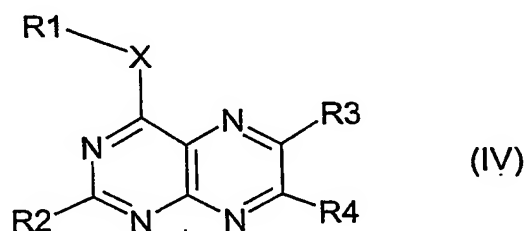
30 thiocarbonyl, hydroxyl, thiol, ether, thio-ether, acetal, thio-acetal, amino, imino, oximino, alkyloximino, aminoacid, cyano, acylamino; thioacylamino; carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic acid ester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or

anhydride or amide, nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl and sulfonamido; or R<sub>2</sub> together with R<sub>3</sub> and the carbon atoms in positions 6 and 7 of the pteridine ring forms a homocyclic or heterocyclic radical;

and/or a pharmaceutically acceptable addition salt thereof and/or a stereoisomer thereof and/or a mono- or a di-N-oxide thereof and/or a solvate thereof and/or a dihydro- or tetrahydropteridine derivative thereof.

The above novel compounds have in common the structural features present in the general formula (I), in particular the pteridine ring is substituted by at least one N,N-containing heterocyclic group being itself N-substituted by a carbonyl or thiocarbonyl or sulfonyl radical or by certain hydrocarbonyl radicals other than C<sub>1-7</sub> alkyl. They also have a potential specific biological activity profile and consequent usefulness in medicinal chemistry as will be detailed below.

In a second embodiment, the present invention relates to a group of novel pteridine derivatives having the general formula (IV):



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wherein X represents an oxygen atom or a group with the formula S(O)<sub>m</sub> wherein m is an integer from 0 to 2, or a group with the formula NZ and wherein:

- R<sub>1</sub> is a group selected from the group consisting of C<sub>1-7</sub> alkyl, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, C<sub>3-10</sub> cycloalkyl, C<sub>3-10</sub> cycloalkenyl, aryl, alkylaryl, arylalkyl, heterocyclic, heterocyclic-substituted alkyl and alkyl-substituted heterocyclic, each of said groups being optionally substituted with one or more substituents independently selected from

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- the group consisting of halogen, C<sub>1-4</sub> alkyl, C<sub>1-4</sub> alkoxy, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, halo C<sub>1-4</sub> alkyl, C<sub>3-10</sub> cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, hydroxyl, sulfhydryl, nitro, hydroxylamino, mercaptoamino, cyano, carboxylic acid or esters or thioesters or amides or thioamides or halides or anhydrides thereof, thiocarboxylic acid or esters or thioesters or amides or thioamides or halides or anhydrides thereof, carbamoyl, thiocarbamoyl, ureido, thio-ureido, amino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; or R<sub>1</sub> is a carboxyalkyl, carboxyaryl, thiocarboxyaryl or thiocarboxyalkyl group;
- Z is a group independently defined as R<sub>1</sub> or Z is hydrogen or the group NZ together with R<sub>1</sub> is either hydroxylamino or an optionally substituted heterocyclic group containing at least one nitrogen atom;
  - R<sub>2</sub> is selected from the group consisting of amino; acylamino; thioacylamino; carbamoyl; thiocarbamoyl, ureido; thioureido, sulfonamido; hydroxylamino; alkoxyamino; thioalkylamino; mercaptoamino, hydrazino; alkylhydrazino; phenylhydrazino; optionally substituted heterocyclic radicals; C<sub>3-7</sub> alkylamino; arylamino; arylalkylamino; cycloalkylamino; alkenylamino; cycloalkenylamino; heterocyclic amino; hydroxyalkylamino; mercaptoalkylamino; C<sub>1-7</sub> alkoxy; C<sub>3-10</sub> cycloalkoxy; thio C<sub>1-7</sub> alkyl; arylsulfoxide; arylsulfone; heterocyclic sulfoxide; heterocyclic sulfone; thio C<sub>3-10</sub> cycloalkyl; aryloxy; arylthio; arylalkyloxy; arylalkylthio; oxyheterocyclic and thioheterocyclic radicals;
  - R<sub>4</sub> is an atom or a group selected from the group consisting of hydrogen; halogen; C<sub>1-7</sub> alkyl; C<sub>2-7</sub> alkenyl; C<sub>2-7</sub> alkynyl; halo C<sub>1-7</sub> alkyl; carboxy C<sub>1-7</sub> alkyl; carboxyaryl; C<sub>1-7</sub> alkoxy; C<sub>3-10</sub> cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; thio C<sub>1-7</sub> alkyl; thio C<sub>3-10</sub> cycloalkyl; thioaryl; thioheterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; mercaptoamino;



acylamino; thio-acylamino; alkoxyamino; thioalkylamino; acetal; thio-  
 acetal; carboxylic acid; carboxylic acid esters, thioesters, halides,  
 anhydrides, amides and thioamides; thiocarboxylic acid; thiocarboxylic  
 acid esters, thioesters, halides, anhydrides, amides and thioamides;  
 5 hydroxyl; sulfhydryl; nitro; cyano; carbamoyl; thiocarbamoyl, ureido;  
 thioureido; alkylamino; cycloalkylamino; alkenylamino; cycloalkenyl-  
 amino; alkynylamino; arylamino; arylalkylamino; hydroxyalkylamino;  
 mercaptoalkylamino; heterocyclic amino; heterocyclic-substituted  
 alkylamino; oximino; alkyloximino; hydrazino; alkylhydrazino;  
 10 phenylhydrazino; cysteinyl acid, esters, thioesters, halides, anhydrides,  
 amides and thioamides thereof; phenyl substituted with one or more  
 substituents independently selected from the group consisting of C<sub>1-7</sub>  
 alkyl, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, halo C<sub>1-7</sub> alkyl, nitro, hydroxyl,  
 sulfhydryl, amino, C<sub>3-10</sub> cycloalkoxy, aryloxy, arylalkyloxy,  
 15 oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub> alkyl, thio  
 C<sub>3-10</sub> cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-  
 substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thio-  
 ureido, sulfon-amido, hydroxylamino, alkoxyamino, mercaptoamino,  
 thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or  
 20 esters or thioesters or halides or anhydrides or amides thereof,  
 thiocarboxylic acid or esters or thioesters or halides or anhydrides or  
 amides thereof, alkylamino, cycloalkylamino, alkenylamino,  
 cycloalkenylamino, alkynylamino, arylamino, arylalkylamino,  
 hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino,  
 25 alkylhydrazino and phenylhydrazino; aryl groups other than phenyl, the  
 said aryl groups being optionally substituted with one or more  
 substituents independently selected from the group consisting of  
 halogen, C<sub>1-7</sub> alkyl, C<sub>1-7</sub> alkoxy, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, halo C<sub>1-7</sub> alkyl,  
 nitro, hydroxyl, sulfhydryl, amino, C<sub>3-10</sub> cycloalkoxy, aryloxy,  
 30 arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub>  
 alkyl, thio C<sub>3-10</sub> cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio,  
 heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl,  
 ureido, thioureido, sulfonamido, hydroxylamino, alkoxyamino,

mercapto-amino, thioalkylamino, acylamino, thioacylamino, cyano,  
 carboxylic acid or esters or thioesters or halides or anhydrides or  
 amides thereof, thiocarboxylic acid or esters or thioesters or halides or  
 anhydrides or amides thereof, alkylamino, cycloalkylamino,  
 5 alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkyl-  
 amino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino,  
 hydrazino, alkylhydrazino and phenylhydrazino; optionally substituted  
 heterocyclic radicals selected from the group consisting of  
 10 oxabicycloheptyl, azabenzimidazolyl, aza-cycloheptyl, azacyclooctyl,  
 azacyclononyl, azabicyclononyl, tetrahydrofuryl, tetrahydropyranyl,  
 tetrahydropyranyl, tetrahydroquinoleinyl, tetrahydrothienyl and dioxide  
 thereof, dihydrothienyl dioxide, dioxindolyl, dioxinyl, dioxenyl,  
 dioxazinyl, thioxanyl, thioxolyl, thiourazolyl, thiotriazolyl, thiopyranyl,  
 thiopyranyl, coumarinyl, quinoleinyl, oxyquinoleinyl, quinuclidinyl,  
 15 xanthinyl, dihydropyranyl, benzodihydrofuryl, benzothiopyranyl,  
 benzothiopyranyl, benzoxazinyl, benzoxazolyl, benzodioxolyl,  
 benzodioxanyl, benzothiadiazolyl, benzotriazinyl, benzothiazolyl,  
 benzoxazolyl, phenothioxinyl, phenothiazolyl, phenothienyl,  
 phenopyranyl, phenoxazolyl, pyridinyl, dihydropyridinyl, tetrahydro-  
 20 pyridinyl, piperidinyl, thiomorpholinyl, pyrazinyl, pyrimidinyl, pyridazinyl,  
 triazinyl, tetrazinyl, triazolyl, benzotriazolyl, tetrazolyl, imidazolyl,  
 pyrazolyl, thiazolyl, thiadiazolyl, isothiazolyl, oxazolyl, oxadiazolyl,  
 pyrrolyl, furyl, dihydrofuryl, furoyl, hydantoinyl, dioxolanyl, dioxolyl,  
 dithianyl, dithienyl, dithiinyl, thienyl, indolyl, indazolyl, benzofuryl,  
 25 quinolyl, quinazoliny, quinoxaliny, carbazolyl, phenoxazinyl,  
 phenothiazinyl, xanthenyl, purinyl, benzothienyl, naphthothienyl,  
 thianthrenyl, pyranyl, pyronyl, benzopyranyl, isobenzofuranyl,  
 chromenyl, phenoxathiinyl, indoliziny, quinoliziny, isoquinolyl,  
 phthalazinyl, naphthiridinyl, cinnoliny, pteridinyl, carboliny, acridiny,  
 30 perimidiny, phenanthroliny, phenazinyl, phenothiazinyl, imidazoliny,  
 imidazolidiny, benzimidazolyl, pyrazoliny, pyrazolidiny, pyrroliny,  
 pyrrolidinyl, piperazinyl, uridinyl, thymidinyl, cytidinyl, aziriny, aziridinyl,  
 diaziriny, diaziridinyl, oxiranyl, oxaziridinyl, dioxiranyl, thiiranyl, azetyl,

dihydroazetyl, azetidiny, oxetyl, oxetanyl, thietyl, thietanyl, diaza-  
 bicyclooctyl, diazetyl, diaziridinonyl, diaziridinethionyl, chromanyl,  
 chromanonyl, thiochromanyl, thiochromanonyl, thiochromenyl,  
 benzofuranyl, benziso-thiazolyl, benzocarbazolyl, benzochromonyl,  
 5 benzisoalloxazinyl, benzocoumarinyl, thiocoumarinyl, phenometoxa-  
 zinyl, phenoparoxazinyl, phentriazinyl, thiodiazinyl, thiodiazolyl, indoxyl,  
 thioindoxyl, benzodiazinyl, phtalidyl, phtalimidinyl, phtalazonyl,  
 alloxazinyl, xanthionyl, isatyl, isopyrazolyl, isopyrazolonyl, urazolyl,  
 urazinyl, uretinyl, uretidinyl, succinyl, succinimido, benzylsultimyl and  
 10 benzylsultamyl; aromatic or heterocyclic substituents substituted with  
 an aliphatic spacer between the pteridine ring and the aromatic or  
 heterocyclic substituent, whereby said aliphatic spacer is a branched or  
 straight, saturated or unsaturated aliphatic chain of 1 to 4 carbon atoms  
 which may contain one or more functions, atoms or radicals  
 15 independently selected from the group consisting of carbonyl (oxo),  
 thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether, acetal, thio-  
 acetal, amino, imino, oximino, alkyloximino, amino-acid, cyano,  
 acylamino, thioacylamino, carbamoyl, thiocarbamoyl, ureido, thio-  
 ureido, carboxylic acid or ester or thioester or halide or anhydride or  
 20 amide, thiocarboxylic acid or ester or thioester or halide or anhydride or  
 amide, nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, hydroxylamino,  
 mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cyclo-  
 alkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxy-  
 alkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino,  
 25 alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and  
 halogen; branched or straight, saturated or unsaturated aliphatic chains  
 of 1 to 7 carbon atoms optionally containing one or more functions,  
 atoms or radicals independently selected from the group consisting of  
 carbonyl (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether,  
 30 acetal, thio-acetal, amino, imino, oximino, alkyloximino, aminoacid,  
 cyano, acylamino, thioacylamino, carbamoyl, thiocarba-moyl, ureido,  
 thioureido, carboxylic acid ester or halide or anhydride or amide,  
 thiocarboxylic acid or ester or thioester or halide or anhydride or amide,

nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; and

5 - R<sub>3</sub> is an atom or a group selected from the group consisting of fluoro, bromo, iodo, C<sub>2-7</sub> alkyl; C<sub>2-7</sub> alkenyl; C<sub>2-7</sub> alkynyl; halo C<sub>1-7</sub> alkyl; C<sub>1-7</sub> alkoxy; C<sub>3-10</sub> cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; thio C<sub>2-7</sub> alkyl; thio C<sub>3-10</sub> cycloalkyl; thioaryl; thioheterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; alkoxyamino; thioalkylamino; mercaptoamino; acylamino; thioacylamino; thio-acetal; carboxylic acid; carboxylic acid esters, thioesters, amides, halides, anhydrides and thioamides; thiocarboxylic acid; thiocarboxylic acid esters, thioesters, amides, halides, anhydrides and thioamides; hydroxyl; sulfhydryl; nitro; carbamoyl; thiocarbamoyl; ureido; thio-ureido; amino; alkylamino; cycloalkylamino; alkenylamino; cycloalkenylamino; alkynylamino; arylamino; arylalkylamino; hydroxyalkyl-amino; mercaptoalkylamino; heterocyclic amino; heterocyclic-substituted alkylamino; oximino; alkylloximino; hydrazino; alkylhydrazino; phenyl-hydrazino; cysteinyl acid, esters, thioesters, amides and thioamides thereof; aryl optionally substituted with one or more substituents independently selected from the group consisting of halogen, C<sub>1-7</sub> alkyl, C<sub>1-7</sub> alkoxy, ω-cyano C<sub>1-7</sub> alkoxy, C<sub>1-7</sub> alkoxy-C<sub>1-7</sub> alkoxy, carboxylic acid ester C<sub>1-7</sub> alkoxy, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, halo C<sub>1-7</sub> alkyl, nitro, hydroxyl, sulfhydryl, amino, C<sub>3-10</sub> cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, carbamoyl, thiocarbamoyl, ureido, thioureido, sulfonamido, hydroxylamino, mercaptoamino, alkoxyamino, thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino,

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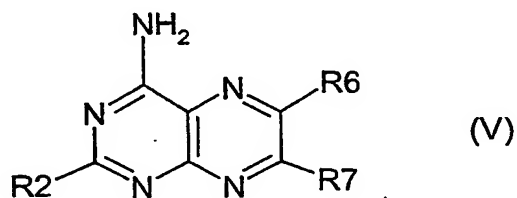
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cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, aryl-  
 amino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, hetero-  
 cyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; optionally  
 substituted heterocyclic radicals; aromatic or heterocyclic substituents  
 5 substituted with an aliphatic spacer between the pteridine ring and the  
 aromatic or heterocyclic substituent, whereby said aliphatic spacer is a  
 branched or straight, saturated or unsaturated aliphatic chain of 1 to 4  
 carbon atoms which may contain one or more functions, atoms or  
 radicals independently selected from the group consisting of carbonyl  
 10 (oxo), thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether, acetal,  
 thio-acetal, amino, imino, oximino, alkyloximino, amino-acid, cyano,  
 carboxylic acid or ester or thioester or amide, nitro, thio C<sub>1-7</sub> alkyl, thio  
 C<sub>3-10</sub> cycloalkyl, alkylamino, cycloalkylamino, alkenylamino, cyclo-  
 alkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkyl-  
 15 amino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkyl-  
 hydrazino, phenylhydrazino, sulfonyl, sulfonamido and halogen;  
 branched or straight, saturated or unsaturated aliphatic chains of 2 to 7  
 carbon atoms optionally containing one or more functions, atoms or  
 radicals independently selected from the group consisting of  
 20 thiocarbonyl, alcohol (hydroxyl), thiol, ether, thio-ether, thio-acetal,  
 amino, imino, oximino, alkyloximino, amino-acid, cyano, acylamino,  
 thioacylamino, carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic  
 acid or ester or thioester or halide or anhydride or amide, thio  
 carboxylic acid or ester or thioester or halide or anhydride or amide,  
 25 nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, hydroxylamino,  
 mercaptoamino, alkylamino, cycloalkylamino, alkenylamino,  
 cycloalkenylamino, alkynylamino, arylamino, arylalkylamino,  
 hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino,  
 alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and  
 30 halogen; or R<sub>3</sub> together with R<sub>4</sub> and the carbon atoms in positions 6 and  
 7 of the pteridine ring forms a homocyclic or heterocyclic radical;

and/or a pharmaceutically acceptable addition salt thereof and/or a stereoisomer thereof and/or a mono- or a di-*N*-oxide thereof and/or a solvate thereof and/or a dihydro- or tetrahydropteridine derivative thereof.

The novel compounds of this second embodiment have in common the structural features present in the general formula (IV), in particular they are at least trisubstituted in positions 2, 4 and 6 of the pteridine ring. They also have a potential specific biological activity profile and consequent usefulness in medicinal chemistry as will be detailed below.

In a third embodiment, the present invention relates to a group of novel 4-amino-pteridine derivatives having the general formula (V):



wherein:

- R<sub>2</sub> is selected from the group consisting of nitrogen-containing heterocyclic radicals other than morpholinyl and piperazinyl, said nitrogen-containing heterocyclic radicals being attached to the pteridine ring by means of a nitrogen atom; benzylamino; phenylethylamino; heterocyclic-substituted alkylamino; aryloxy; arylthio; arylsulfonyl; arylalkyloxy; arylalkylthio; C<sub>1-7</sub> alkylsulfonyl; heterocyclic-substituted alkyloxy; and heterocyclic-substituted alkylthio;
- R<sub>6</sub> and R<sub>7</sub> are independently selected from the group consisting of hydrogen; halogen; C<sub>1-7</sub> alkyl; C<sub>2-7</sub> alkenyl; C<sub>2-7</sub> alkynyl; halo C<sub>1-7</sub> alkyl; carboxy C<sub>1-7</sub> alkyl; C<sub>1-7</sub> alkylsulfonyl; carboxyaryl; C<sub>1-7</sub> alkoxy; C<sub>3-10</sub> cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; C<sub>1-7</sub> alkylthio; thio C<sub>3-10</sub> cycloalkyl; arylthio; arylsulfonyl; thio-heterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; mercaptoamino; acylamino; thioacylamino; alkoxyamino; thioalkylamino; acetal; thio-acetal; carboxylic acid; carboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; thiocarboxylic acid; thiocarboxylic acid esters, thioesters,

halides, anhydrides, amides and thioamides; hydroxyl; sulfhydryl; nitro; cyano; carbamoyl; thiocarbamoyl; ureido; thioureido; amino; alkyl-amino; cycloalkylamino; alkenylamino; cycloalkenylamino; alkynyl-amino; arylamino; arylalkylamino; hydroxyalkylamino; mercaptoalkyl-amino; heterocyclic amino; heterocyclic-substituted alkylamino; oximino; alkyloximino; hydrazino; alkylhydrazino; phenylhydrazino; cysteinyl acid, esters, thioesters, halides, anhydrides, amides and thioamides thereof; aryl optionally substituted with one or more substituents independently selected from the group consisting of halogen, C<sub>1-7</sub> alkyl, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, halo C<sub>1-7</sub> alkyl, nitro, hydroxyl, sulfhydryl, amino, C<sub>1-7</sub> alkoxy, C<sub>3-10</sub> cycloalkoxy, aryloxy, arylalkyloxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, thioaryl, thio-heterocyclic, arylalkylthio, heterocyclic-substituted alkylthio, formyl, C<sub>1-7</sub> alkanoyl (acyl), carbamoyl, thiocarbamoyl, ureido, thioureido, sulfonamido, hydroxyl-amino, alkoxyamino, mercaptoamino, thioalkylamino, acylamino, thioacylamino, cyano, carboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, thiocarboxylic acid or esters or thioesters or halides or anhydrides or amides thereof, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino; optionally substituted heterocyclic radicals; aryl or heterocyclic radicals substituted with an aliphatic spacer between the pteridine ring and said aryl or heterocyclic radical, whereby said aliphatic spacer is a branched or straight, saturated or unsaturated aliphatic chain of 1 to 4 carbon atoms which may contain one or more functions, atoms or radicals independently selected from the group consisting of carbonyl, thiocarbonyl, hydroxyl, thiol, ether, thioether, acetal, thioacetal, amino, imino, oximino, alkyloximino, amino-acid, cyano, acylamino, thioacyl-amino, carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic acid or ester or thioester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C<sub>1-7</sub> alkyl,

thio C<sub>3-10</sub> cycloalkyl, hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and halogen; branched or straight, saturated or unsaturated aliphatic chains of 1 to 7 carbon atoms optionally containing one or more functions, atoms or radicals independently selected from the group consisting of halogen, carbonyl, thiocarbonyl, hydroxyl, thiol, ether, thio-ether, acetal, thio-acetal, amino, imino, oximino, alkyloximino, aminoacid, cyano, acylamino, thioacylamino, carbamoyl, thiocarbamoyl, ureido, thioureido, carboxylic acid ester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, hydroxylamino, mercapto-amino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl and sulfonamido; or R<sub>6</sub> together with R<sub>7</sub> and the carbon atoms in positions 6 and 7 of the pteridine ring forms a homocyclic or heterocyclic radical;

and/or a pharmaceutically acceptable addition salt thereof and/or a stereoisomer thereof and/or a mono- or a di-*N*-oxide thereof and/or a solvate thereof and/or a dihydro- or tetrahydropteridine derivative thereof.

The novel compounds of this third embodiment have in common the structural features present in the general formula (V), in particular they are amino-substituted at position 4 of the pteridine ring and substituted at position 2 of the pteridine ring by a radical being a nitrogen-containing or oxygen-containing or sulfur-containing nucleophile. They also have a potential specific biological activity profile and consequent usefulness in medicinal chemistry as will be detailed below.

In a further embodiment, the present invention relates to the unexpected finding that at least one desirable biological property such as, but not limited to, the ability to decrease the proliferation of lymphocytes, or to



decrease T-cell activation, or to decrease B-cell or monocytes or macrophages activation, or to inhibit the release of certain cytokines, or in inhibiting human TNF- $\alpha$  production is a feature which is present in the said groups of novel compounds. As a consequence, the invention relates to pharmaceutical compositions comprising as an active principle at least one pteridine derivative having the general formula (I) and/or at least one pteridine derivative having the general formula (IV), and/or at least one 4-amino-pteridine derivative having the general formula (V) and/or a pharmaceutically acceptable addition salt thereof and/or a stereoisomer thereof and/or a mono- or a di-*N*-oxide thereof and/or a solvate and/or a dihydro- or tetrahydro-pteridine derivative thereof.

Compounds having the general formulae (I), (IV) and (V) are highly active immunosuppressive agents, antineoplastic agents, anti-allergic agents or anti-viral agents which, together with one or more pharmaceutically acceptable carriers, may be formulated into pharmaceutical compositions for the prevention or treatment of pathologic conditions such as, but not limited to, immune and autoimmune disorders, organ and cells transplant rejections, allergic conditions, cell proliferative disorders, cardiovascular disorders, disorders of the central nervous system and viral diseases. Compounds having the general formulae (I), (IV) and (V) are also useful for the prevention or treatment of a TNF- $\alpha$ -related disorder in a mammal, such as for instance:

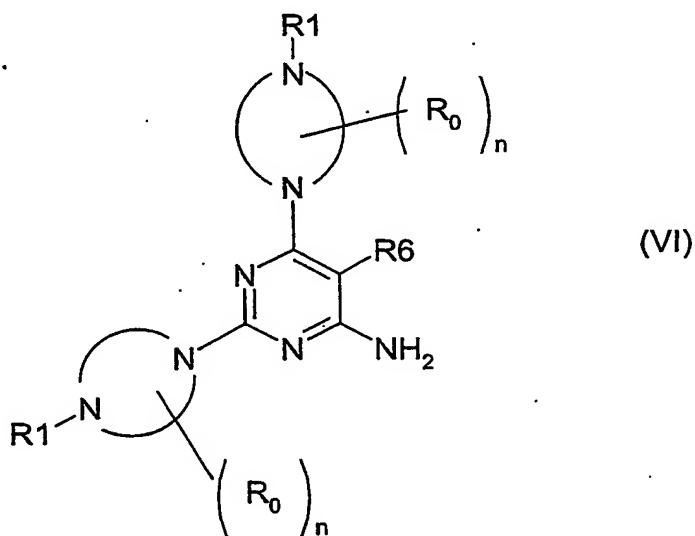
- septic or endotoxic shock,
- TNF- $\alpha$ - mediated diseases,
- pathologies and conditions associated with and/or induced by abnormal levels of TNF- $\alpha$  occurring in a systemic, localized or particular tissue type or location in the body of the mammal,
- toxic effects of TNF- $\alpha$  and/or anti-cancer chemotherapeutic agents,
- injuries after irradiation of a tissue of the mammal by radio-elements, and
- cachexia.

In a further embodiment, the present invention relates to combined preparations containing at least one compound of the general formula (I), (IV) or (V) and one or more drugs such as immunosuppressant and/or immunomodulator drugs, antineoplastic drugs, anti-histamines, inhibitors of

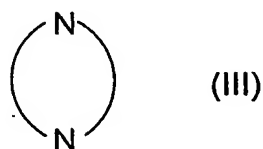
agents causative of allergic conditions, or antiviral agents. In a further embodiment, the present invention relates to the prevention or treatment of the above-cited pathologic conditions by administering to the patient in need thereof an effective amount of a compound of the general formula (I), (IV) or (V) optionally in the form of a pharmaceutical composition or combined preparation with another suitable drug.

In another embodiment, the present invention relates to various processes and methods for making the novel pteridine derivatives defined in general formulae (I), (IV) and (V), as well as their pharmaceutically acceptable salts, N-oxides, solvates, enantiomers and dihydro- and tetrahydroderivatives.

In another embodiment, the present invention relates to a family of novel polysubstituted 6-aminopyrimidines having the general formula (VI):

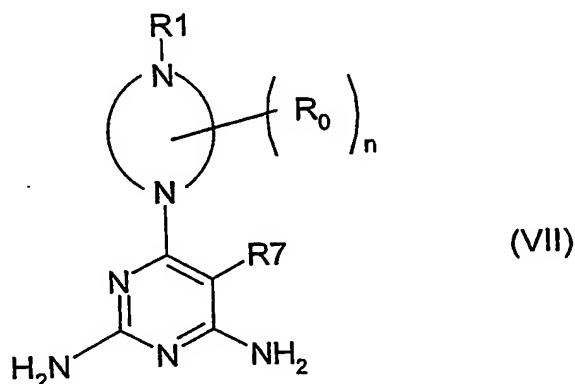


wherein each of  $n$ ,  $R_0$  and

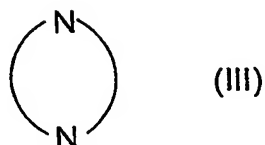


are as defined hereinabove with respect to formula (II); wherein  $R_1$  is as defined hereinabove with respect to formula (II) or is hydrogen; and wherein  $R_6$  is selected from the group consisting of nitro and amino.

In another embodiment, the present invention relates to a family of novel polysubstituted 2,6-diaminopyrimidines having the general formula (VII):



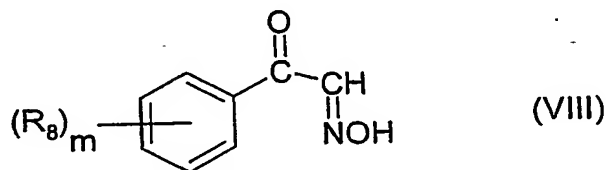
wherein:



and  $R_0$ ,  $R_1$  and  $n$  are as defined hereinabove with respect to formula (II), and  
 5 wherein  $R_7$  is selected from the group consisting of hydrogen, nitroso and amino.

The said novel polysubstituted pyrimidines having the general formulae (VI) and (VII) are useful as intermediates for making some of the pteridine derivatives of the present invention.

10 In another embodiment, the present invention relates to a family of novel substituted phenylglyoxalmonoximes having the general formula (VIII):



wherein  $m$  is from 0 to 5, and wherein each substituent  $R_8$  is independently selected from the group consisting of halogen, cyano, piperidino, imidazol-1-yl, hydroxy, amino, protected amino (such as acetamido), nitro, benzoxy,  
 15 acetoxo,  $C_{1-7}$  alkoxy and  $C_{1-7}$  alkyl, as well as a method for making them from  $R_8$ -substituted acetophenones. The said novel substituted phenylglyoxalmonoximes having the general formula (VIII) are useful as intermediates for

making some of the pteridine derivatives defined in the general formulae (I), (IV) and (V) of the present invention.

In yet another embodiment, the present invention relates to a family of novel 2-substituted-4,6-diamino-5-nitrosopyrimidines and 2-substituted-4,5,6-triaminopyrimidines, as well as a method for making them, which are useful as intermediates for making some of the pteridine derivatives of the present invention.

#### BRIEF DESCRIPTION OF THE DRAWINGS

10        Figure 1 schematically shows a first method for making trisubstituted and tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II).

Figure 2 schematically shows a second method for making trisubstituted and tetrasubstituted pteridine derivatives having the formula (I) 15 wherein  $R_5$  is a group having the formula (II).

Figure 3 schematically shows a third method for making trisubstituted and tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II).

Figure 4 schematically shows a fourth method for making trisubstituted 20 and tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II).

Figure 5 schematically shows a fifth method for making trisubstituted and tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II).

25        Figure 6 schematically shows a sixth method for making trisubstituted and tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II).

Figure 7 schematically shows a seventh method for making trisubstituted and tetrasubstituted pteridine derivatives having the formula (I) 30 wherein  $R_5$  is a group having the formula (II).

Figure 8 schematically shows a method for making trisubstituted and tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_4$  and  $R_5$  are identical groups having the formula (II).

Figure 9 schematically shows a method for making 2,4,6-trisubstituted pteridine derivatives having the formula (IV) wherein  $R_3$  is a phenyl group substituted by a nitrogen-containing function.

Figure 10 schematically shows a method for making 2,4,6-trisubstituted pteridine derivatives having the formula (IV) wherein  $R_3$  is a phenyl group substituted by an oxygen-containing function.

Figures 11 and 12 represent alternative schemes for preparing 2,4,6-trisubstituted pteridine derivatives according to this invention.

Figure 13 represents a scheme for preparing 2,4,7-trisubstituted pteridine derivatives according to this invention.

Figure 14 represents a scheme for preparing unsymmetrical 2,4,6-trisubstituted pteridines and 2,4,7-trisubstituted pteridines according to this invention.

Figure 15 represents a scheme for preparing symmetrical 2,4,6-trisubstituted pteridines and 2,4,7-trisubstituted pteridines according to this invention.

## DEFINITIONS

Unless otherwise stated herein, the term "trisubstituted" means that three of the carbon atoms being in positions 2, 4 and 6 or, alternatively, in positions 2, 4 and 7 of the pteridine ring (according to standard atom numbering for the pteridine ring) are substituted with an atom or group other than hydrogen. The term "tetrasubstituted" means that all four carbon atoms being in positions 2, 4, 6 and 7 of the pteridine ring are substituted with an atom or group other than hydrogen.

As used herein with respect to a substituting radical, and unless otherwise stated, the term "C<sub>1-7</sub> alkyl" means straight and branched chain saturated acyclic hydrocarbon monovalent radicals having from 1 to 7 carbon atoms such as, for example, methyl, ethyl, propyl, n-butyl, 1-methylethyl (isopropyl), 2-methylpropyl (isobutyl), 1,1-dimethylethyl (ter-butyl), 2-methyl-

butyl, n-pentyl, dimethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, n-heptyl and the like.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " acyl " broadly refers to a carbonyl (oxo) group adjacent to a C<sub>1-7</sub> alkyl radical, a C<sub>3-10</sub> cycloalkyl radical, an aryl radical, an arylalkyl radical or a heterocyclic radical, all of them being such as herein defined; representative examples include acetyl, benzoyl, naphthoyl and the like; similarly, the term " thioacyl " refers to a C=S (thioxo) group adjacent to one of the said radicals.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " C<sub>1-7</sub> alkylene " means the divalent hydrocarbon radical corresponding to the above defined C<sub>1-7</sub> alkyl, such as methylene, bis(methylene), tris(methylene), tetramethylene, hexamethylene and the like.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " C<sub>3-10</sub> cycloalkyl " means a mono- or polycyclic saturated hydrocarbon monovalent radical having from 3 to 10 carbon atoms, such as for instance cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl and the like, or a C<sub>7-10</sub> polycyclic saturated hydrocarbon monovalent radical having from 7 to 10 carbon atoms such as, for instance, norbornyl, fenchyl, trimethyltricycloheptyl or adamantyl.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " C<sub>3-10</sub> cycloalkyl-alkyl " refers to an aliphatic saturated hydrocarbon monovalent radical (preferably a C<sub>1-7</sub> alkyl such as defined above) to which a C<sub>3-10</sub> cycloalkyl (such as defined above) is already linked such as, but not limited to, cyclohexylmethyl, cyclopentylmethyl and the like.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " N-acyl substituted alkyl " refers to a carbonyl group linked to a divalent aliphatic radical (preferably a C<sub>1-7</sub> alkylene such as above defined) on one side and to the nitrogen atom of an N-containing group (such as alkylamino, arylamino or heterocyclic amino) on the other side.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " C<sub>3-10</sub> cycloalkylene " means the divalent hydrocarbon radical corresponding to the above defined C<sub>3-10</sub> cycloalkyl.

As used herein with respect to a substituting radical, and unless  
5 otherwise stated, the term " aryl " designate any mono- or polycyclic aromatic monovalent hydrocarbon radical having from 6 to 30 carbon atoms such as but not limited to phenyl, naphthyl, anthracenyl, phenanthracenyl, fluoranthenyl, chrysenyl, pyrenyl, biphenylyl, terphenyl, picenyl, indenyl, biphenyl, indacenyl, benzocyclobutenyl, benzocyclooctenyl and the like, including fused benzoC<sub>4-8</sub>  
10 cycloalkyl radicals (the latter being as defined above) such as, for instance, indanyl, tetrahydronaphthyl, fluorenyl and the like, each of said radicals being optionally substituted with one or more substituents independently selected from the group consisting of halogen, amino, trifluoromethyl, hydroxyl, sulfhydryl and nitro, such as for instance 4-fluorophenyl, 4-chlorophenyl, 3,4-  
15 dichlorophenyl, 4-cyanophenyl, 2,6-dichlorophenyl, 2-fluorophenyl, 3-chlorophenyl, 3,5-dichlorophenyl and the like.

As used herein; e.g. with respect to a substituting radical such as the combination of substituents in positions 6 and 7 of the pteridine ring together with the carbon atoms in positions 6 and 7 of the pteridine ring, and unless  
20 otherwise stated, the term " homocyclic " means a mono- or polycyclic, saturated or mono-unsaturated or polyunsaturated hydrocarbon radical having from 4 up to 15 carbon atoms but including no heteroatom in the said ring; for instance said combination of substituents in positions 6 and 7 of the pteridine ring may form a C<sub>2-6</sub> alkylene radical, such as tetramethylene, which cyclizes  
25 with the carbon atoms in positions 6 and 7 of the pteridine ring.

As used herein with respect to a substituting radical (including a combination of substituents in positions 6 and 7 of the pteridine ring together with the carbon atoms in positions 6 and 7 of the pteridine ring), and unless  
30 otherwise stated, the term " heterocyclic " means a mono- or polycyclic, saturated or mono-unsaturated or polyunsaturated monovalent hydrocarbon radical having from 2 up to 15 carbon atoms and including one or more heteroatoms in one or more heterocyclic rings, each of said rings having from 3 to 10 atoms (and optionally further including one or more heteroatoms

attached to one or more carbon atoms of said ring, for instance in the form of a carbonyl or thiocarbonyl or selenocarbonyl group, and/or to one or more heteroatoms of said ring, for instance in the form of a sulfone, sulfoxide, N-oxide, phosphate, phosphonate or selenium oxide group), each of said

5 heteroatoms being independently selected from the group consisting of nitrogen, oxygen, sulfur, selenium and phosphorus, also including radicals wherein a heterocyclic ring is fused to one or more aromatic hydrocarbon rings for instance in the form of benzo-fused, dibenzo-fused and naphto-fused heterocyclic radicals; within this definition are included heterocyclic radicals

10 such as, but not limited to, diazepinyl, oxadiazinyl, thiadiazinyl, dithiazinyl, triazolonyl, diazepinonyl, triazepinyl, triazepinonyl, tetrazepinonyl, benzo-quinolinyl, benzothiazinyl, benzothiazinonyl, benzoxa-thiinyl, benzodioxinyl, benzodithiinyl, benzoxazepinyl, benzothiazepinyl, benzodiazepinyl, benzodioxepinyl, benzodithiepinyl, benzoxazocinyl, benzothiazocinyl,

15 benzodiazocinyl, benzoxathiocinyl, benzodioxocinyl, benzotrioxepinyl, benzoxathiazepinyl, benzoxadiazepinyl, benzothiadiazepinyl, benzotriazepinyl, benzoxathiepinyl, benzotriazinonyl, benzoxazolinonyl, azetidiny, azaspirooundecyl, dithiaspirodecyl, selenazinyl, selenazolyl, selenophenyl, hypoxanthinyl, azahypoxanthinyl, bipyrazinyl, bipyridinyl, oxazolidinyl,

20 diselenopyrimidinyl, benzodioxocinyl, benzopyrenyl, benzopyranonyl, benzophenazinyl, benzoquinoliziny, dibenzocarbazolyl, dibenzoacridinyl, dibenzophenazinyl, dibenzothiepinyl, dibenzooxepinyl, dibenzopyranonyl, dibenzoquinoxalinyl, dibenzothiazepinyl, dibenzoisoquinolinyl, tetraaza-adamantyl, thiatetraazaadamantyl, oxauracil, oxazinyl, dibenzothiophenyl,

25 dibenzofuranyl, oxazoliny, oxazolonyl, azaindolyl, azolonyl, thiazoliny, thiazolonyl, thiazolidinyl, thiazanyl, pyrimidonyl, thiopyrimidonyl, thiamorpholinyl, azlactonyl, naphtindazolyl, naphtindolyl, naphtothiazolyl, naphtothioxolyl, naphtoxindolyl, naphtotriazolyl, naphtopyranyl, oxabicycloheptyl, azabenzimidazolyl, azacycloheptyl, azacyclooctyl, azacyclononyl, azabicyclononyl,

30 tetrahydrofuryl, tetrahydropyranyl, tetrahydropyronyl, tetrahydroquinoleinyl, tetrahydrothienyl and dioxide thereof, dihydrothienyl dioxide, dioxindolyl, dioxinyl, dioxenyl, dioxazinyl, thioxanyl, thioxolyl, thiourazolyl, thiotriazolyl, thiopyranyl, thiopyronyl, coumarinyl, quinoleinyl, oxyquinoleinyl, quinuclidinyl,



xanthinyl, dihydropyranyl, benzodihydrofuryl, benzothiopyranyl, benzothio-  
 pyranyl, benzoxazinyl, benzoxazolyl, benzodioxolyl, benzodioxanyl,  
 benzothiadiazolyl, benzotriazinyl, benzothiazolyl, benzoxazolyl, pheno-  
 thioxinyl, phenothiazolyl, phenothienyl (benzothiofuranlyl), phenopyranyl,  
 5 phenoxazolyl, pyridinyl, dihydropyridinyl, tetrahydropyridinyl, piperidinyl,  
 morpholinyl, thiomorpholinyl, pyrazinyl, pyrimidinyl, pyridazinyl, triazinyl,  
 tetrazinyl, triazolyl, benzotriazolyl, tetrazolyl, imidazolyl, pyrazolyl, thiazolyl,  
 thiadiazolyl, isothiazolyl, oxazolyl, oxadiazolyl, pyrrolyl, furyl, dihydrofuryl,  
 furoyl, hydantoinyl, dioxolanyl, dioxolyl, dithianyl, dithienyl, dithiinyl, thienyl,  
 10 indolyl, indazolyl, benzofuryl, quinolyl, quinazolinyl, quinoxalinyl, carbazolyl,  
 phenoxazinyl, phenothiazinyl, xanthenyl, purinyl, benzothienyl, naphthothienyl,  
 thianthrenyl, pyranyl, pyronyl, benzopyranyl, isobenzofuranlyl, chromenyl,  
 phenoxathiinyl, indolizinyl, quinolizinyl, isoquinolyl, phthalazinyl, naphthiridinyl,  
 cinnolinyl, pteridinyl, carbolinyl, acridinyl, perimidinyl, phenanthrolinyl,  
 15 phenazinyl, phenothiazinyl, imidazolynyl, imidazolidinyl, benzimidazolyl,  
 pyrazolinyl, pyrazolidinyl, pyrrolinyl, pyrrolidinyl, piperazinyl, uridinyl,  
 thymidinyl, cytidinyl, azirinyl, aziridinyl, diazirinyl, diaziridinyl, oxiranyl,  
 oxaziridinyl, dioxiranyl, thiiranyl, azetyl, dihydroazetyl, azetidynyl, oxetyl,  
 oxetanyl, thietyl, thietanyl, diazabicyclooctyl, diazetyl, diaziridinonyl,  
 20 diaziridinethionyl, chromanyl, chromanonyl, thiochromanyl, thiochromanonyl,  
 thiochromenyl, benzofuranlyl, benzisothiazolyl, benzocarbazolyl, benzo-  
 chromonyl, benzisoalloxazinyl, benzocoumarinyl, thiocoumarinyl,  
 phenometoxazinyl, phenoparoxazinyl, phentriazinyl, thio-diazinyl, thiodiazolyl,  
 indoxyl, thioindoxyl, benzodiazinyl (e.g. phtalazinyl), phtalidyl, phtalimidinyl,  
 25 phtalazonyl, alloxazinyl, dibenzopyranyl (i.e. xanthonyl), xanthionyl, isatyl,  
 isopyrazolyl, isopyrazolonyl, urazolyl, urazinyl, uretinyl, uretidinyl, succinyl,  
 succinimido, benzylsultimyl, benzylsultamyl and the like, including all possible  
 isomeric forms thereof, wherein each carbon atom of said heterocyclic ring  
 may be independently substituted with a substituent selected from the group  
 30 consisting of halogen, nitro, C<sub>1-7</sub> alkyl (optionally containing one or more  
 functions or radicals selected from the group consisting of carbonyl (oxo),  
 alcohol (hydroxyl), ether (alkoxy), acetal, amino, imino, oximino, alkyloximino,  
 amino-acid, cyano, carboxylic acid ester or amide, nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-</sub>

10 cycloalkyl, C<sub>1-7</sub> alkylamino, cycloalkylamino, alkenylamino, cycloalkenyl-  
 amino, alkynylamino, arylamino, arylalkylamino, hydroxylalkylamino,  
 mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino,  
 phenylhydrazino, sulfonyl, sulfonamido and halogen), C<sub>3-7</sub> alkenyl, C<sub>2-7</sub>  
 5 alkynyl, halo C<sub>1-7</sub> alkyl, C<sub>3-10</sub> cycloalkyl, aryl, arylalkyl, alkylaryl, alkylacyl,  
 arylacyl, hydroxyl, amino, C<sub>1-7</sub> alkylamino, cycloalkylamino, alkenylamino,  
 cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkyl-  
 amino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino,  
 phenylhydrazino, sulfhydryl, C<sub>1-7</sub> alkoxy, C<sub>3-10</sub> cycloalkoxy, aryloxy, arylalkyl-  
 10 oxy, oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub>  
 cycloalkyl, thioaryl, thioheterocyclic, arylalkylthio, heterocyclic-substituted  
 alkylthio, formyl, hydroxylamino, cyano, carboxylic acid or esters or thioesters  
 or amides thereof, thiocarboxylic acid or esters or thioesters or amides  
 thereof; depending upon the number of unsaturations in the 3 to 10 membered  
 15 ring, heterocyclic radicals may be sub-divided into heteroaromatic (or  
 "heteroaryl ") radicals and non-aromatic heterocyclic radicals; when a  
 heteroatom of the said non-aromatic heterocyclic radical is nitrogen, the latter  
 may be substituted with a substituent selected from the group consisting of C<sub>1-7</sub>  
 7 alkyl, C<sub>3-10</sub> cycloalkyl, aryl, arylalkyl and alkylaryl.

20 As used herein with respect to a substituting radical, and unless  
 otherwise stated, the terms " C<sub>1-7</sub> alkoxy ", " C<sub>3-10</sub> cycloalkoxy ", " aryloxy ",  
 " arylalkyloxy ", " oxyheterocyclic ", " thio C<sub>1-7</sub> alkyl ", " thio C<sub>3-10</sub> cycloalkyl ",  
 " arylthio ", " arylalkylthio " and " thioheterocyclic " refer to substituents  
 wherein a C<sub>1-7</sub> alkyl radical, respectively a C<sub>3-10</sub> cycloalkyl, aryl, arylalkyl or  
 25 heterocyclic radical (each of them such as defined herein), are attached to an  
 oxygen atom or a divalent sulfur atom through a single bond, such as but not  
 limited to methoxy, ethoxy, propoxy, butoxy, pentoxy, isopropoxy, sec-butoxy,  
 tert-butoxy, isopentoxy, cyclopropyloxy, cyclobutyloxy, cyclopentyloxy,  
 thiomethyl, thioethyl, thiopropyl, thiobutyl, thiopentyl, thiocyclopropyl,  
 30 thiocyclobutyl, thiocyclopentyl, thiophenyl, phenyloxy, benzyloxy,  
 mercaptobenzyl, cresoxy, and the like.

As used herein with respect to a substituting atom, and unless otherwise stated, the term halogen means any atom selected from the group consisting of fluorine, chlorine, bromine and iodine.

As used herein with respect to a substituting radical, and unless  
5 otherwise stated, the term " halo C<sub>1-7</sub> alkyl " means a C<sub>1-7</sub> alkyl radical (such as above defined) in which one or more hydrogen atoms are independently replaced by one or more halogens (preferably fluorine, chlorine or bromine), such as but not limited to difluoromethyl, trifluoromethyl, trifluoroethyl, octafluoropentyl, dodecafluoroheptyl, dichloromethyl and the like.

10 As used herein with respect to a substituting radical, and unless otherwise stated, the terms " C<sub>2-7</sub> alkenyl " designate a straight and branched acyclic hydrocarbon monovalent radical having one or more ethylenic unsaturations and having from 2 to 7 carbon atoms such as, for example, vinyl, 1-propenyl, 2-propenyl (allyl), 1-butenyl, 2-butenyl, 2-pentenyl, 3-  
15 pentenyl, 3-methyl-2-butenyl, 3-hexenyl, 2-hexenyl, 2-heptenyl, 1,3-butadienyl, pentadienyl, hexadienyl, heptadienyl, heptatrienyl and the like, including all possible isomers thereof.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " C<sub>3-10</sub> cycloalkenyl " mean a monocyclic mono- or  
20 polyunsaturated hydrocarbon monovalent radical having from 3 to 8 carbon atoms, such as for instance cyclopropenyl, cyclobutenyl, cyclopentenyl, cyclopentadienyl, cyclohexenyl, cyclohexadienyl, cycloheptenyl, cycloheptadienyl, cycloheptatrienyl, cyclooctenyl, cyclooctadienyl and the like, or a C<sub>7-10</sub> polycyclic mono- or polyunsaturated hydrocarbon mono-valent radical having  
25 from 7 to 10 carbon atoms such as dicyclopentadienyl, fencheryl (including all isomers thereof, such as  $\alpha$ -pinolenyl), bicyclo[2.2.1]hept-2-enyl, bicyclo[2.2.1]hepta-2,5-dienyl, cyclo-fencheryl and the like.

As used herein with respect to a substituting radical, and unless otherwise stated, the term " C<sub>2-7</sub> alkynyl " defines straight and branched chain  
30 hydrocarbon radicals containing one or more triple bonds and optionally at least one double bond and having from 2 to 7 carbon atoms such as, for example, acetylenyl, 1-propynyl, 2-propynyl, 1-butyne, 2-butyne, 2-pentyne,

1-pentynyl, 3-methyl-2-butyryl, 3-hexynyl, 2-hexynyl, 1-penten-4-ynyl, 3-penten-1-ynyl, 1,3-hexadien-1-ynyl and the like.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms "arylalkyl", "arylalkenyl" and "heterocyclic-substituted alkyl" refer to an aliphatic saturated or ethylenically unsaturated hydrocarbon monovalent radical (preferably a C<sub>1-7</sub> alkyl or C<sub>2-7</sub> alkenyl radical such as defined above) onto which an aryl or heterocyclic radical (such as defined above) is already bonded, and wherein the said aliphatic radical and/or the said aryl or heterocyclic radical may be optionally substituted with one or more substituents independently selected from the group consisting of C<sub>1-4</sub> alkyl, trifluoromethyl, halogen, amino, nitro, hydroxyl, sulfhydryl and nitro, such as but not limited to benzyl, 4-chlorobenzyl, 2-fluorobenzyl, 4-fluorobenzyl, 3,4-dichlorobenzyl, 2,6-dichlorobenzyl, 4-*ter*-butylbenzyl, 3-methylbenzyl, 4-methylbenzyl, phenylpropyl, 1-naphtylmethyl, phenylethyl, 1-amino-2-phenylethyl, 1-amino-2-[4-hydroxy-phenyl]ethyl, 1-amino-2-[indol-2-yl]ethyl, styryl, pyridylmethyl (including all isomers thereof), pyridylethyl, 2-(2-pyridyl)isopropyl, oxazolylbutyl, 2-thienylmethyl, pyrrolylethyl, morpholinylethyl, imidazol-1-yl-ethyl, benzodioxolylmethyl and 2-furylmethyl.

As used herein with respect to a substituting radical, and unless otherwise stated, the term "alkylaryl" and "alkyl-substituted heterocyclic" refer to an aryl or heterocyclic radical (such as defined above) onto which are bonded one or more aliphatic saturated hydrocarbon mono-valent radicals, preferably one or more C<sub>1-7</sub> alkyl or C<sub>3-10</sub> cycloalkyl radicals as defined above such as, but not limited to, *o*-toluyl, *m*-toluyl, *p*-toluyl, 2,3-xylyl, 2,4-xylyl, 3,4-xylyl, *o*-cumenyl, *m*-cumenyl, *p*-cumenyl, *o*-cymenyl, *m*-cymenyl, *p*-cymenyl, mesityl, *ter*-butylphenyl, lutidinyl (i.e. dimethylpyridyl), 2-methylaziridinyl, methylbenzimidazolyl, methylbenzofuranyl, methylbenzothiazolyl, methylbenzotriazolyl, methylbenzoxazolyl and methylbenzselenazolyl.

As used herein with respect to a substituting radical, and unless otherwise stated, the term "alkoxyaryl" refers to an aryl radical (such as defined above) onto which is (are) bonded one or more C<sub>1-7</sub> alkoxy radicals as defined above, preferably one or more methoxy radicals, such as, but not

limited to, 2-methoxyphenyl, 3-methoxyphenyl, 4-methoxyphenyl, 3,4-dimethoxyphenyl, 2,4,6-trimethoxyphenyl, methoxynaphtyl and the like.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms "alkylamino", "cycloalkylamino", "alkenyl-amino", "cycloalkenylamino", "arylamino", "arylalkylamino", "heterocyclic amino", "hydroxyalkylamino", "mercaptoalkylamino" and "alkynylamino" mean that respectively one (thus monosubstituted amino) or even two (thus disubstituted amino) C<sub>1-7</sub> alkyl, C<sub>3-10</sub> cycloalkyl, C<sub>2-7</sub> alkenyl, C<sub>3-10</sub> cycloalkenyl, aryl, arylalkyl, heterocyclic, mono- or polyhydroxy C<sub>1-7</sub> alkyl, mono- or polymercapto C<sub>1-7</sub> alkyl or C<sub>2-7</sub> alkynyl radical(s) (each of them as defined herein, respectively) is/are attached to a nitrogen atom through a single bond or, in the case of heterocyclic, include a nitrogen atom, such as but not limited to, anilino, benzylamino, methylamino, dimethylamino, ethylamino, diethylamino, isopropylamino, propenylamino, n-butylamino, ter-butylamino, dibutylamino, morpholinoalkylamino, morpholiny, piperidiny, piperaziny, hydroxymethylamino,  $\beta$ -hydroxyethylamino and ethynylamino; this definition also includes mixed disubstituted amino radicals wherein the nitrogen atom is attached to two such radicals belonging to two different sub-set of radicals, e.g. an alkyl radical and an alkenyl radical, or to two different radicals within the same sub-set of radicals, e.g. methylethylamino; among disubstituted amino radicals, symetrically substituted are more easily accessible and thus usually preferred.

As used herein with respect to a substituting radical, and unless otherwise stated, the terms "(thio)carboxylic acid ester", "(thio)carboxylic acid thioester" and "(thio)carboxylic acid amide" refer to radicals wherein the carboxyl or thiocarboxyl group is directly attached to the pteridine ring (e.g. in the 6- and/or 7-position) and wherein said carboxyl or thiocarboxyl group is bonded to the hydrocarbonyl residue of an alcohol, a thiol, a polyol, a phenol, a thiophenol, a primary or secondary amine, a polyamine, an amino-alcohol or ammonia, the said hydrocarbonyl residue being selected from the group consisting of alkyl, alkenyl, alkynyl, cycloalkyl, cycloalkenyl, aryl, arylalkyl, alkylaryl, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino,

arylamino, arylalkylamino, heterocyclic amino, hydroxyalkylamino, mercapto-alkylamino or alkynylamino (such as above defined, respectively).

As used herein with respect to a substituting radical, and unless otherwise stated, the term " amino-acid " refers to a radical derived from a molecule having the chemical formula  $H_2N-CHR-COOH$ , wherein R is the side group of atoms characterizing the amino-acid type; said molecule may be one of the 20 naturally-occurring amino-acids or any similar non naturally-occurring amino-acid.

As used herein and unless otherwise stated, the term " stereoisomer " refers to all possible different isomeric as well as conformational forms which the compounds of formula (I), (IV) and (V) may possess, in particular all possible stereochemically and conformationally isomeric forms, all diastereomers, enantiomers and/or conformers of the basic molecular structure. Some compounds of the present invention may exist in different tautomeric forms, all of the latter being included within the scope of the present invention.

As used herein and unless otherwise stated, the term " enantiomer " means each individual optically active form of a compound of the invention, having an optical purity or enantiomeric excess (as determined by methods standard in the art) of at least 80% (i.e. at least 90% of one enantiomer and at most 10% of the other enantiomer), preferably at least 90% and more preferably at least 98%.

As used herein and unless otherwise stated, the term " solvate " includes any combination which may be formed by a pteridine derivative of this invention with a suitable inorganic solvent (e.g. hydrates) or organic solvent, such as but not limited to alcohols, ketones, esters and the like.

As used herein and unless otherwise stated, the terms " dihydro-pteridine derivative " and " tetrahydropteridine derivative " refer to the hydrogenation products of the pteridine derivatives having the general formula (I), i.e. derivatives wherein two hydrogen atoms are present in positions 5 and 6, or 7 and 8, of the pteridine ring, or wherein four hydrogen atoms are present in positions 5, 6, 7 and 8 of the said ring; such hydrogenated derivatives are easily accessible from the pteridine derivatives using hydrogenation methods well known in the art.

## DETAILED DESCRIPTION OF THE INVENTION

A first object of the invention is to provide a pharmaceutical composition having high immunosuppressive activity. Thus, the present invention relates in particular to the medical applications of a group of pteridine derivatives, their pharmaceutically acceptable salts, N-oxides, solvates, polymorphs, dihydro- and tetrahydroderivatives and enantiomers, possessing unexpectedly desirable pharmaceutical properties, in particular which are highly active immunosuppressive agents, and as such are useful in the treatment in transplant rejection and/or in the treatment of certain inflammatory diseases.

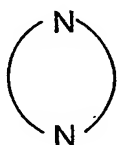
Surprisingly, the compounds of the present invention show a broader therapeutic spectrum profile than merely immunosuppressive activity, as is evidenced by the results obtained in the diversity of test procedures disclosed hereinbelow. A further advantageous feature of the compounds of the present invention resides in their excellent oral activity.

In the first embodiment of the invention, the novel pteridine derivatives are as defined in the general formula (I), wherein each of the substituents  $R_0$ ,  $R_1$ ,  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  may independently correspond to any of the definitions given above, in particular with any of the individual meanings (such as illustrated above) of generic terms used for substituting radicals such as, but not limited to, "C<sub>1-7</sub> alkyl", "C<sub>3-10</sub> cycloalkyl", "C<sub>2-7</sub> alkenyl", "C<sub>2-7</sub> alkynyl", "aryl", "homocyclic", "heterocyclic", "halogen", "C<sub>3-10</sub> cycloalkenyl", "alkylaryl", "arylalkyl", "alkylamino", "cycloalkylamino", "alkenylamino", "alkynylamino", "arylamino", "arylalkylamino", "heterocyclic amino", "hydroxyalkylamino", "mercaptoalkylamino", "alkynylamino", "C<sub>1-7</sub> alkoxy", "C<sub>3-10</sub> cycloalkoxy", "thio C<sub>1-7</sub> alkyl", "thio C<sub>3-10</sub> cycloalkyl", "halo C<sub>1-7</sub> alkyl", "amino-acid" and the like.

Stereoisomers of the compounds of this invention may be formed by using reactants in their single enantiomeric form wherever possible in the manufacturing process or by resolving the mixture of stereoisomers by conventional methods. One such method is liquid chromatography using one or more suitable chiral stationary phases including, for example, polysaccharides, in particular cellulose or amylose derivatives. Commercially

available polysaccharide-based chiral stationary phases are ChiralCel™ CA, OA, OB, OC, OD, OF, OG, OJ and OK, and Chiralpak™ AD, AS, OP(+) and OT(+). Appropriate eluents or mobile phases for use in combination with said polysaccharide-based chiral stationary phases are hydrocarbons such as hexane and the like, optionally admixed with an alcohol such as ethanol, isopropanol and the like. The above mixture of enantiomers may alternatively be separated by making use of microbial resolution or by resolving the diastereoisomeric salts formed with chiral acids such as mandelic acid, camphorsulfonic acid, tartaric acid, lactic acid and the like or with chiral bases such as brucine and the like. The resolving agent may be cleaved from the separated diastereoisomers, e.g. by treatment with acids or bases, in order to generate the pure enantiomers of the compounds of the invention. Conventional resolution methods were compiled e.g. by Jaques et al. in "Enantiomers, Racemates and Resolution" (Wiley Interscience, 1981).

In the general formula (II), the schematic notation



preferably means a heterocyclic group selected from the group consisting of:

- piperazin-1-yl,
- 4-imidazolin-1-yl,
- imidazolidin-1-yl,
- 2,3-dihydropyrazol-1-yl,
- 2,3,4,5-tetrahydropyrazol-1-yl,
- 3-pyrazolin-1-yl,
- 4-pyrazolin-1-yl,
- pyrazolidin-1-yl,
- 2,3-dihydropyrazin-1-yl,
- tetrahydropyrazin-1-yl,
- dihydropyrimidin-1-yl,
- tetrahydropyrimidin-1-yl,
- dihydropyridazin-1-yl,



- tetrahydropyridazin-1-yl.
- hexahydropyridazin-1-yl,
- dihydrofurazan-2-yl,
- tetrahydrofurazan-2-yl,
- 5 - dihydrophenazin-5-yl,
- dihydrotriazol-1-yl,
- dihydrotriazol-2-yl,
- tetrahydrotriazol-1-yl,
- tetrahydrotriazol-2-yl,
- 10 - dihydrotriazin-1-yl,
- tetrahydrotriazin-1-yl,
- tetrahydrooxadiazin-2-yl,
- tetrahydrothiadiazin-2-yl,
- dihydroindazol-1-yl,
- 15 - dihydroindazol-2-yl,
- tetrahydrophtalazin-2-yl,
- tetrahydrophtalazin-3-yl,
- tetrahydroquinoxalin-1-yl,
- tetrahydroquinazolin-1-yl,
- 20 - tetrahydroquinazolin-3-yl,
- dihydrocinnolin-1-yl,
- dihydrocinnolin-2-yl,
- tetrahydrocinnolin-1-yl,
- tetrahydrocinnolin-2-yl,
- 25 - dihydroperimidin-1-yl,
- tetrahydrodiazepin-1-yl, and
- oxides, sulfones and selenium oxides of the latter.

In the general formula (II), the said heterocyclic group may be substituted, at one or more carbon atoms, by a number  $n$  of substituents  $R_0$  wherein  $n$  is an integer from 0 to 6 and wherein, when  $n$  is at least 2, each  $R_0$  may be defined  
 30 independtly from the others. The presence of one or more substituents  $R_0$  is a way for introducing chirality into the pteridine derivatives having the general formula (I) as well as into the polysubstituted 6-aminopyrimidines having the

general formula (IV) and the polysubstituted 2,6-diaminopyrimidines having the general formula (V). In practice, the choice of substituents  $R_0$  may be restricted by the commercial availability of the substituted heterocyclic amine, depending upon the specific nature of the heterocyclic group.



5 More preferably the schematic notation

represents a piperazin-1-yl group, in which case preferably  $n$  is 0, 1 or 2, and a representative example of the substituent  $R_0$  is methyl or phenyl (such as for instance in 2-methylpiperazin-1-yl, 2-phenylpiperazin-1-yl and 2,5-dimethylpiperazin-1-yl).

10 As shown in the general formula (II), a requirement of the invention is that one of the two nitrogen atoms of the heterocyclic ring bears a substituent  $R_1$  which has a carbonyl (oxo) or thiocarbonyl (thioxo) or sulfonyl function immediately adjacent to the said nitrogen atom. In other words, this means that when  $R_1$  is selected from, respectively, acyl, thioacyl, amide, thioamide,  
15 sulfonyl, sulfinyl, carboxylate and thiocarboxylate, then  $R_1$  together with the nitrogen atom to which it is attached forms, respectively, an amide, thioamide, urea, thiourea, sulfonamido, sulfinamido, carbamato or thiocarbamato group.

As already specified above, one or more of the substituents  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  of the pteridine ring may be a group represented by the general  
20 formula (II). As will be apparent from the synthetic routes described hereinafter, preferably one or two substituents of the pteridine ring are groups independently having the general formula (II). More preferably these substituents are  $R_4$  and  $R_5$ , i.e. the ones in positions 2 and/or 4 of the pteridine ring. When both positions 2 and 4 of the pteridine ring are substituted by  
25 groups having the general formula (II), these substituents may be the same (as shown in figure 8) or different (as shown in figures 6 and 7).

As already specified above, the remaining positions of the pteridine ring, i.e. the ones which are not substituted by a group represented by the general formula (II), may either be unsubstituted (i.e. the relevant groups  $R_2$ ,

R<sub>3</sub>, R<sub>4</sub> or R<sub>5</sub> are hydrogen) or be substituted independently from each other. Preferably one or two of the remaining positions, more preferably selected from positions 2, 6 and 7, of the pteridine ring are substituted. When two remaining positions (e.g. positions 2 and 6) of the pteridine ring are substituted, the relevant substituents are preferably different from each other.

Some preferred pteridine derivatives having the general formula (I) according to the invention are more specifically illustrated in the following examples and defined in the following claims. For instance, useful pteridine species disclosed below include those wherein:

- 10 - R<sub>2</sub> is selected from the group consisting of hydrogen, C<sub>1-7</sub> alkyl, C<sub>3-10</sub> cycloalkyl, aryl and heteroaryl, preferably a phenyl group optionally substituted with one or more substituents selected from the group consisting of halogen, C<sub>1-7</sub> alkyl and C<sub>1-7</sub> alkoxy, more preferably p-fluorophenyl, p-chlorophenyl, p-tolyl, p-acetamidophenyl or 3,4-
- 15 dimethoxyphenyl, and/or
- R<sub>3</sub> is selected from the group consisting of hydrogen, C<sub>1-7</sub> alkyl, C<sub>3-10</sub> cycloalkyl, aryl and heteroaryl, preferably hydrogen, and/or
- R<sub>4</sub> is amino or a group represented by the general formula (II), and/or
- R<sub>5</sub> is a group selected from the group consisting of piperazin-1-yl, 2-
- 20 methylpiperazin-1-yl, 2-phenylpiperazin-1-yl and 2,5-dimethylpiperazin-1-yl, the said group being substituted in the 4 position with a substituent R<sub>1</sub> which has a carbonyl (oxo) or thiocarbonyl (thioxo) or sulfonyl function (e.g. R<sub>1</sub> is selected from the group consisting of acyl, thioacyl, amide, thioamide, sulfonyl, sulfinyl, carboxylate and thiocarboxylate).

25 Especially useful species of pteridine derivatives having the general formula (I) are those wherein R<sub>5</sub> is a piperazin-1-yl group substituted in the 4 position with a substituent R<sub>1</sub>, wherein R<sub>1</sub> is selected from the group consisting of:

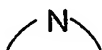
- COR<sub>8</sub> wherein R<sub>8</sub> is selected from the group consisting of hydrogen; C<sub>1-7</sub> alkyl; C<sub>3-10</sub> cycloalkyl; aryl optionally substituted with one or more substituents selected from the group consisting of halogen, C<sub>1-7</sub> alkyl, cyano and C<sub>1-7</sub> alkoxy; heterocyclic optionally substituted with one or more halogen atoms; arylalkyl; aryloxyalkyl; arylalkoxyalkyl; alkoxyalkyl;
- 30

arylalkoxy; aryloxy; arylalkenyl; heterocyclic-substituted alkyl; alkylamino and arylamino; representative but non limiting examples of  $R_8$  are methyl, ethyl, pentyl, cyclohexyl, phenyl, 4-fluorophenyl, 4-chlorophenyl, 3,4-dichlorophenyl, 4-butylphenyl, 4-cyanophenyl, 2-methoxyphenyl, 3-methoxyphenyl, 4-pentoxyphenyl, naphthyl, 2-thienyl, 4-pyridinyl, 1-tetrahydropyrrolyl, 2-tetrahydropyrrolyl, 2-furanyl, 3-furanyl, 2,4-dichloro-5-fluoro-3-pyridinyl, diethylamino, diisopropylamino, diphenylamino, phenylethyl, 4-chlorobenzyl, phenoxyethyl, benzyloxyethyl, methoxyethyl, 2-thienylethyl, styryl, benzyloxy, phenoxy, 1-amino-2-phenylethyl, 1-amino-2-[4-hydroxyphenyl]ethyl and 1-amino-2-[indol-2-yl]ethyl,

- $CSR_9$ , wherein  $R_9$  is selected from the group consisting of alkylamino and aryloxy, such as but not limited to dimethylamino and phenoxy, and
- $SO_2R_{10}$ , wherein  $R_{10}$  is selected from the group consisting of aryl and arylalkyl, such as but not limited to phenyl and benzyl.

The present invention further provides various processes and methods for making the novel pteridine derivatives having the general formula (I). As a general rule, the preparation of these compounds is based on the principle that, starting from a suitable pteridine precursor (a diaminopyrimidine), each of the substituents  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  may be introduced separately (except, of course, when  $R_2$  together with  $R_3$  forms a homocyclic or heterocyclic radical) without adversely influencing the presence of one or more substituents already introduced at other positions on the pteridine ring or the capacity to introduce further substituents later on.

Methods of manufacture have been developed by the present inventors which may be used alternatively to, or may be combined with, the methods of synthesis already known in the art of pteridine derivatives (depending upon the targeted final compound). For instance, methods for simultaneously introducing  $R_2$  and  $R_3$  in the form of a homocyclic or heterocyclic radical at positions 6 and 7 of the pteridine ring are already known from U.S. Patent No. 2,581,889. The synthesis of mono- and di-*N*-oxides of the pteridine derivatives of this invention can easily be achieved by treating the said derivatives with an oxidizing agent such as, but not limited to, hydrogen peroxide (e.g. in the



presence of acetic acid) or a peracid such as chloroperbenzoic acid. Dihydro- and tetrahydropteridine derivatives of this invention can easily be obtained by catalytic hydrogenation of the corresponding pteridine derivatives, e.g. by placing the latter in a hydrogen atmosphere in the presence of platinum oxide or platinum. The methods for making the pteridine derivatives of the present invention will now be explained in more details by reference to the appended figures 1 to 8 wherein, unless otherwise stated hereinafter, each of the substituting groups or atoms  $R_2$ ,  $R_3$ ,  $R_4$  and  $R_5$  is as defined in formula (I) of the summary of the invention and, more specifically, may correspond to any of the individual meanings disclosed above. For a reason of convenience, each figure shows piperazin-1-yl as a representative example of the heterocyclic ring schematically represented as in the general formula (II), however it should be understood that the methods of the invention are not particularly limited to piperazin-1-yl but can be applied successfully to any other heterocyclic ring meeting the requirements specified hereinabove.

In the description of the reaction steps involved in each figure, reference is made to the use of certain catalysts and/or certain types of solvents. It should be understood that each catalyst mentioned should be used in a catalytic amount well known to the skilled person with respect to the type of reaction involved. Solvents that may be used in the following reaction steps include various kinds of organic solvents such as protic solvents, polar aprotic solvents and non-polar solvents as well as aqueous solvents which are inert under the relevant reaction conditions. More specific examples include aromatic hydrocarbons, chlorinated hydrocarbons, ethers, aliphatic hydrocarbons, alcohols, esters, ketones, amides, water or mixtures thereof, as well as supercritical solvents such as carbon dioxide (while performing the reaction under supercritical conditions). The suitable reaction temperature and pressure conditions applicable to each kind of reaction step will not be detailed herein but do not depart from the relevant conditions already known to the skilled person with respect to the type of reaction involved and the type of solvent used (in particular its boiling point).

Figure 1 schematically shows a first method for making trisubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II) and wherein  $R_2$  or  $R_3$  is hydrogen, as well as tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II). In step (a), a nitroso group is introduced on position 5 of the pyrimidine ring of a 2- $R_4$ -substituted 4-oxo-6-aminopyrimidine by using sodium nitrite under aqueous acidic conditions. Reduction of the nitroso group in step (b) is achieved either catalytically ( $Pt/H_2$ ) in the presence of a protic solvent, or chemically using sodium dithionite or ammonium sulfide in water. Then in a next step (c), condensing the resulting 2- $R_4$ -substituted 4-oxo-5,6-diaminopyrimidine with an  $\alpha$ -ketoaldoxime bearing a radical  $R_2$ , wherein  $R_2$  may be *inter alia*  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl or heteroaryl, in a protic solvent such as methanol under acidic conditions regioselectively yields a 4-oxopteridine bearing a  $R_4$  substituent in position 2 and a  $R_2$  substituent in position 6 of the pteridine ring. Alternatively, a 2- $R_4$ -substituted 4-oxo-7- $R_3$ -substituted pteridine derivative can be obtained in step (d) by reacting the 2- $R_4$ -substituted 4-oxo-5,6-diamino-pyrimidine with a monosubstituted glyoxal bearing the group  $R_3$ , wherein  $R_3$  may be *inter alia*  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl or heteroaryl, under neutral or basic conditions. Alternatively, a 2- $R_4$ -substituted-4-oxo-6,7-disubstituted pteridine derivative can be obtained in step (e) by reacting the 2- $R_4$ -substituted 4-oxo-5,6-diamino-pyrimidine with a disubstituted glyoxal bearing groups  $R_2$  and  $R_3$ , wherein each of  $R_2$  and  $R_3$  is independently selected (i.e.  $R_2$  and  $R_3$  may be identical or different) from the group consisting of  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. Activation of the (tautomeric) hydroxyl substituent in position 4 of the pteridine ring for a nucleophilic displacement reaction occurs in step (f) by preparing the corresponding 4-[(1,2,4)-triazolyl] pteridine derivative, e.g. using  $POCl_3$  or 4-chlorophenyl phosphorodichloridate and 1,2,4-triazole in pyridine as solvent. When  $R_4$  is an amino group, protection of  $R_4$  may further be necessary before carrying out this reaction. The amino group can be protected for instance by an acetyl group, which can be hydrolysed back to the amino group in a next step. Nucleophilic substitution is performed in step (g) by mixing the triazolyl pteridine derivative with piperazine at room temperature in

a polar aprotic solvent such as 1,4-dioxane. Coupling of the piperazin-1-yl substituent in position 4 of the pteridine ring with the desired carboxylic acid or thiocarboxylic acid chloride or sulfonyl chloride  $R_1Cl$  occurs in step (h) at room temperature in a solvent such as pyridine.

5 Figure 2 schematically shows a second method for making trisubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II) and wherein  $R_2$  or  $R_3$  is hydrogen, as well as tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II). In step (a), the diazonium salt of p-chloroaniline is first formed by  
 10 using sodium nitrite under aqueous acidic conditions and then reacted with a 2- $R_4$ -substituted 4-chloro-6-amino-pyrimidine to yield an azo intermediate. In step (b), the chlorine atom in position 4 of the pyrimidinyl ring is replaced by a piperazinyl group. Reductive cleavage of the azo compound then yields the corresponding 2- $R_4$ -substituted 4-(piperazin-1-yl)-5,6-diaminopyrimidine in  
 15 step (c). Condensation of the latter with an  $\alpha$ -ketoaldoxime bearing a radical  $R_2$ , wherein  $R_2$  may be *inter alia*  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl or heteroaryl, in a protic solvent such as methanol under acidic conditions leads in step (d) to the formation of a 2- $R_4$ -substituted 4-(piperazin-1-yl)-6- $R_2$ -substituted pteridine. Alternatively, a 2- $R_4$ -substituted 4-(piperazin-1-yl)-7- $R_3$ -substituted  
 20 pteridine derivative can be obtained in step (e) by reacting the 2- $R_4$ -substituted 4-(piperazin-1-yl)-5,6-diaminopyrimidine with a monosubstituted glyoxal bearing the group  $R_3$ , wherein  $R_3$  may be *inter alia*  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl or heteroaryl, under neutral or basic conditions. Alternatively, a 2- $R_4$ -substituted-4-(piperazin-1-yl)-6,7-disubstituted pteridine derivative can be  
 25 obtained in step (f) by reacting the 2- $R_4$ -substituted 4-(piperazin-1-yl)-5,6-diamino-pyrimidine with a disubstituted glyoxal bearing groups  $R_2$  and  $R_3$ , wherein each of  $R_2$  and  $R_3$  is independently selected (i.e.  $R_2$  and  $R_3$  may be identical or different) from the group consisting of  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. Coupling of the  
 30 piperazin-1-yl group with an acid or sulfonyl chloride  $R_1Cl$  occurs in the last step (g) as in the first method above.

Figure 3 schematically shows a third method for making trisubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the

formula (II) and wherein  $R_2$  or  $R_3$  is hydrogen, as well as tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II). This method starts from the pyrimidine derivative obtained after step (b) of the first method above. Formation of the pteridine ring occurs in

5 step (b) through reaction of the said pyrimidine derivative with a suitable  $\alpha$ -ketoaldoxime bearing a radical  $R_2$ , wherein  $R_2$  may be *inter alia*  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl or heteroaryl, in a protic solvent such as methanol under acidic conditions. Alternatively, a 2- $R_4$ -substituted 4-oxo-7- $R_3$ -substituted pteridine derivative can be obtained in step (c) by reacting the 2- $R_4$ -

10 substituted 4-oxo-5,6-diaminopyrimidine with a monosubstituted glyoxal bearing the group  $R_3$ , wherein  $R_3$  may be *inter alia*  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl or heteroaryl, under neutral or basic conditions. Alternatively, a 2- $R_4$ -substituted 4-oxo-6,7-disubstituted pteridine derivative can be obtained in step (d) by reacting the 2- $R_4$ -substituted 4-oxo-5,6-diamino-pyrimidine with a

15 disubstituted glyoxal bearing groups  $R_2$  and  $R_3$ , wherein each of  $R_2$  and  $R_3$  is independently selected (i.e.  $R_2$  and  $R_3$  may be identical or different) from the group consisting of  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. A piperazin-1-yl group is then directly introduced, in step (e), at position 4 of the pteridine ring by reaction of the 2- $R_4$ -substituted

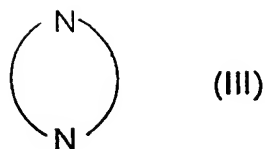
20 4-oxopteridine with piperazine and 1,1,1,3,3,3-hexamethyldisilazane as a reagent. Final coupling step (f) of the piperazin-1-yl group with an acid or sulfonyl chloride  $R_1Cl$  then proceeds as in the first method above.

Figure 4 schematically shows a fourth method for making trisubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the

25 formula (II) and wherein  $R_2$  or  $R_3$  is hydrogen, as well as tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II). In this method,  $R_4$  preferably is amino. In a first step (a), the chlorine atom at position 4 of the pyrimidine ring of a 2- $R_4$ -substituted 4-chloro-6-aminopyrimidine is displaced by an appropriate  $R_1$ -monosubstituted

30 piperazine wherein  $R_1$  may be acyl or sulfonyl, thus e.g. yielding a novel polysubstituted 2,6-diaminopyrimidine having the general formula (V) wherein  $n = 0$ ,  $R_7$  is hydrogen and the heterocyclic ring having the formula (III)





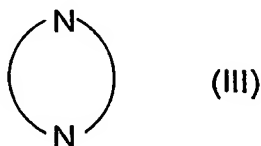
is a piperazinyl group.

Many monosubstituted piperazines required for step (a) are commercially available, such as for instance:

- 1-(2-furoyl) piperazine,
- 5 - 1-(4-chlorobenzenesulfonyl) piperazine ,
- 1-(4-fluorobenzoyl) piperazine,
- 1-(4-methoxyphenylsulfonyl) piperazine,
- 1-(ethoxycarbonyl) piperazine,
- 1-(tetrahydro-2-furoyl) piperazine,
- 10 - 1-(thien-2-ylcarbonyl) piperazine,
- 1-[(2,3-dihydro-1,4-benzodioxin-2-yl)carbonyl] piperazine,
- 1-acetylpiperazine,
- 1-formylpiperazine, and
- 1-methanesulfonylpiperazine.

- 15 Monosubstituted piperazines which are not commercially available may easily be prepared by reacting piperazine with any commercially available acid chloride or sulfonyl chloride.

- 20 Introduction of a nitroso group at position 5 of the pyrimidine ring occurs in step (b) under aqueous acidic conditions in the presence of sodium nitrite, thus e.g. yielding a novel polysubstituted 2,6-diaminopyrimidine having the general formula (V) wherein  $n = 0$ ,  $R_7$  is nitroso and the heterocyclic ring having the formula (III)



is a piperazinyl group.

- 25 Reduction of the nitroso functionality of this intermediate into a free amino group is then effected in step (c) by means of reducing agents such as

$\text{Na}_2\text{S}_2\text{O}_4$  or  $(\text{NH}_4)_2\text{S}$  in water, or catalytically ( $\text{Pt}/\text{H}_2$ ) in the presence of a protic solvent, thus e.g. yielding a novel polysubstituted 2,5,6-triaminopyrimidine having the general formula (V) wherein  $n = 0$ ,  $\text{R}_7$  is amino and the heterocyclic ring having the general formula (III)

5 is a piperazinyl group.

In order to regioselectively obtain a 2,4,6-trisubstituted pteridine derivative, the substituted 5,6-diaminopyrimidine is then reacted in step (d) with an  $\alpha$ -ketoaldoxime bearing the group  $\text{R}_2$ , wherein  $\text{R}_2$  may be *inter alia*  $\text{C}_{1-7}$  alkyl,  $\text{C}_{3-10}$  cycloalkyl, aryl or heteroaryl, under acidic conditions in the  
10 presence of a solvent such as methanol. Alternatively, a 2,4,7-trisubstituted pteridine derivative can be obtained in step (f) by reacting the substituted 5,6-diaminopyrimidine with a monosubstituted glyoxal bearing the group  $\text{R}_3$ , wherein  $\text{R}_3$  may be *inter alia*,  $\text{C}_{1-7}$  alkyl,  $\text{C}_{3-10}$  cycloalkyl, aryl or heteroaryl, under neutral or basic conditions. Alternatively, a 2,4,6,7-tetrasubstituted  
15 pteridine derivative can be obtained in step (e) by reacting the substituted 5,6-diaminopyrimidine with a disubstituted glyoxal bearing the groups  $\text{R}_2$  and  $\text{R}_3$ , wherein each of  $\text{R}_2$  and  $\text{R}_3$  is independently selected (i.e.  $\text{R}_2$  and  $\text{R}_3$  may be identical or different) from the group consisting of  $\text{C}_{1-7}$  alkyl,  $\text{C}_{3-10}$  cycloalkyl, aryl and heteroaryl, under neutral or basic conditions.

20 Figure 5 schematically shows a fifth method for making trisubstituted pteridine derivatives having the formula (I) wherein  $\text{R}_5$  is a group having the formula (II) and wherein  $\text{R}_3$  is hydrogen. In particular, figure 5 shows a scheme making use of a coupling compound such as a carboxylic or sulfonic acid having the general formula  $\text{R}_{11}\text{ZOOH}$ , wherein  $\text{Z}$  is selected from the  
25 group consisting of the carbon atom and the  $\text{SO}$  group, and wherein  $\text{R}_{11}$  is a group selected from the group consisting of  $\text{C}_{1-7}$  alkyl,  $\text{C}_{3-10}$  cycloalkyl, aryl, heteroaryl and alkylaryl, wherein the said group  $\text{R}_{11}$  is optionally substituted with one or more substituents selected from the group consisting of halogen, amino and protected amino groups, and wherein the said substituent may be  
30 at any position (such as the  $\alpha$  position or the  $\omega$  position) with respect to the carboxylic or sulfonic acid group. In particular, the said coupling compound may be a carboxylic acid  $\text{R}_{11}\text{CO}_2\text{H}$  or a sulfonic acid  $\text{R}_{11}\text{SO}_3\text{H}$  or a preferably

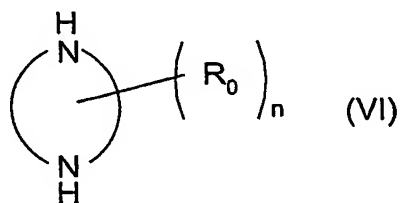
amino-protected naturally-occurring amino-acid (e.g. L-alanine, L-phenylalanine, L-tyrosine, L-proline, L-tryptophane, L-leucine, L-isoleucine, L-lysine, L-valine, glycine, L-histidine, L-serine, L-arginine, L-aspartic acid, L-cysteine or L-glutamine) or synthetic non naturally-occurring amino-acid. This method provides coupling, in one single step (a), of the said compound to the second nitrogen atom of a heterocyclic ring having the general formula (III) and being substituted at position 4 of a pteridine ring. That is, this method starts for instance from a pteridine intermediate such as obtained after step (g) of the scheme shown in figure 1, or after any of steps (d), (e) and (f) of the scheme shown in figure 2, or after step (e) of the scheme shown in figure 3. When the coupling compound is a carboxylic acid  $R_{11}CO_2H$ , the said pteridine intermediate is reacted with the coupling compound preferably in an aprotic solvent, such as dimethyl-formamide or dichloromethane or mixtures thereof, and in the presence of a suitable coupling reagent, such as 1,3-dicyclohexylcarbodiimide or 1,3-diisopropylcarbodiimide or diisopropylethylamine or o-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium tetrafluoroborate. As is usual for this kind of coupling reaction, all free amino groups of the amino-acids are most preferably protected before carrying out the coupling reaction. When the amino-acid has two or more amino groups, the protecting groups for the said amino groups may be the same or different. A few examples of amino-protecting groups are benzyloxycarbonyl (which may be introduced by reaction of the desired amino-acid with benzylchloroformate under alkaline conditions, e.g. making use of sodium hydroxide or hydrogenocarbonate) and 9-fluorenylmethoxycarbonyl (which may be introduced by reaction of the desired amino-acid with 9-fluorenylmethyl chloroformate). Another example of an amino-protecting group is a tert-butoxycarbonyl group which may be introduced by reaction of the desired amino-acid with di-tert-butyl dicarbonate under alkaline conditions. Other suitable amino-protecting groups include triphenylmethyl (trityl) and trifluoroacetyl groups. First, the amino-protected amino-acid is coupled to the second nitrogen atom of the heterocyclic ring e.g. by using any method conventional in peptide synthesis. Finally, in order to afford the desired (4-

substituted-piperazin-1-yl) pteridine derivative, the amino-protecting group is removed by deprotection methods conventional in the art, such as:

- when the amino-protecting group is a phenylmethoxycarbonyl group, cleavage of the benzylic ether function by hydrogenolysis, e.g. using H<sub>2</sub>, Pd-C at about 25°C, or under strongly acidic conditions (e.g. making use of bromhydric acid), or
- when the amino-protecting group is a tert-butoxycarbonyl group, by treatment with an acid, e.g. using aqueous hydrochloric acid or trifluoroacetic acid, under conditions mild enough to avoid further cleavage of the molecule, or
- when the amino-protecting group is a 9-fluorenylmethoxycarbonyl group, by treatment with a base such as piperidine.

Figure 6 schematically shows a sixth method for making trisubstituted pteridine derivatives having the formula (I) wherein R<sub>5</sub> is a group having the formula (II) and wherein R<sub>3</sub> or R<sub>2</sub> is hydrogen, as well as tetrasubstituted pteridine derivatives having the formula (I) wherein R<sub>5</sub> is a group having the formula (II). In step (a), the thiol function of 2-mercapto-4,6-diaminopyrimidine is alkylated, preferably methylated by reaction with methyl iodide in the presence of a solvent such as ethanol, in order to yield 2-thiomethyl-4,6-diaminopyrimidine. Introduction of a nitroso group in the 5-position of the pyrimidine ring is then achieved in step (b) by using sodium nitrite under aqueous acidic conditions. In step (c), the methylthio group in the 2-position is exchanged for a group R<sub>4</sub> by reaction with an appropriate nucleophile, wherein R<sub>4</sub> is as defined above and preferably is primary or secondary amino, C<sub>1-7</sub> alkoxy, aryloxy, C<sub>3-10</sub> cycloalkoxy, heteroaryloxy, mercapto C<sub>1-7</sub>alkyl, mercaptoaryl, mercapto C<sub>3-10</sub>cycloalkyl or mercapto-heteroaryl. Reduction of the nitroso group is then achieved in step (d) either catalytically (Pt/H<sub>2</sub>) in the presence of a protic solvent or chemically using sodium dithionite or ammonium sulfide in the presence of water. Then in step (e), the resulting 2-R<sub>4</sub>-substituted-4,5,6-triaminopyrimidine is condensed, under acidic conditions in the presence of a solvent such as methanol, with an α-ketoaldoxime bearing the group R<sub>2</sub>, wherein R<sub>2</sub> may be C<sub>1-7</sub> alkyl, C<sub>3-10</sub> cycloalkyl, aryl or heteroaryl, into a 2,6-substituted-4-aminopteridine derivative. Alternatively, the

corresponding 2,7-substituted-4-aminopteridine derivative can be obtained in step (f) by reacting the 2- $R_4$ -substituted-4,5,6-triaminopyrimidine with a monosubstituted glyoxal bearing a group  $R_3$ , wherein  $R_3$  may be inter alia  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl or heteroaryl. Alternatively, a 2- $R_4$ -substituted 4-amino-6,7-disubstituted pteridine derivative can be obtained in step (g) by reacting the 2- $R_4$ -substituted 4,5,6-triaminopyrimidine with a disubstituted glyoxal bearing groups  $R_2$  and  $R_3$ , wherein each of  $R_2$  and  $R_3$  is independently selected (i.e.  $R_2$  and  $R_3$  may be identical or different) from the group consisting of  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. In step (h), acidic or basic hydrolysis of the amino group at position 4 of the pteridine ring is performed and results in the corresponding 4-oxopteridine derivative. In step (i), the hydroxyl group of the tautomeric form of the latter is activated by nucleophilic displacement, e.g. by preparing the 4-[(1,2,4)-triazolyl] pteridine derivative. Finally in a first part of step (j), a nucleophilic displacement is performed by mixing the said 4-triazolylpteridine derivative with a nucleophile having the general formula (VI):



wherein  $R_0$  and  $n$  are as already defined above with respect to formula (II).

Since this nucleophile, and optionally also the nucleophile used in step (c), has a heterocyclic ring containing at least two nitrogen atoms, the second nitrogen atom of each heterocyclic ring can be acylated or sulfonylated in a last part of step (j) by treating the intermediate with an appropriate acid chloride or sulfonyl chloride  $R_1\text{Cl}$  in a aprotic solvent such as dimethylformamide, pyridine or dichloromethane and, if necessary, in the presence of a base such as a tertiary amine (e.g. triethylamine).

Figure 7 schematically shows a seventh method for making trisubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a group having the formula (II) and wherein  $R_3$  or  $R_2$  is hydrogen, as well as tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_5$  is a

group having the formula (II), starting from the 2-thiomethyl-5-nitroso-4,6-diaminopyrimidine obtained after step (b) of the scheme shown in figure 6. Reduction of the nitroso group is achieved in step (a) either catalytically (Pt/H<sub>2</sub>) in the presence of a protic solvent or chemically using sodium dithionite or ammonium sulfide in the presence of water. Then in step (b), 2-thiomethyl-4,5,6-triaminopyrimidine is condensed, under acidic conditions in the presence of a solvent such as methanol, with an  $\alpha$ -ketoaldoxime bearing the group R<sub>2</sub>, wherein R<sub>2</sub> may be *inter alia* C<sub>1-7</sub> alkyl, C<sub>3-10</sub> cycloalkyl, aryl or heteroaryl, thus regioselectively yielding a 2-thiomethyl-4-amino-6-R<sub>2</sub>-substituted-pteridine derivative. Alternatively, the corresponding 2-thiomethyl-4-amino-7-R<sub>3</sub>-substituted pteridine is obtained in step (c) by reacting 2-thiomethyl-4,5,6-triamino-pyrimidine with a monosubstituted glyoxal bearing a group R<sub>3</sub>, wherein R<sub>3</sub> may be *inter alia* C<sub>1-7</sub> alkyl, C<sub>3-10</sub> cycloalkyl, aryl or heteroaryl. Alternatively, the corresponding 2-thiomethyl-4-amino-6-R<sub>2</sub>-7-R<sub>3</sub>-substituted pteridine is obtained in step (d) by reacting 2-thiomethyl-4,5,6-triamino-pyrimidine with a disubstituted glyoxal bearing groups R<sub>2</sub> and R<sub>3</sub>, wherein each of R<sub>2</sub> and R<sub>3</sub> is independently selected (i.e. R<sub>2</sub> and R<sub>3</sub> may be identical or different) from the group consisting of C<sub>1-7</sub> alkyl, C<sub>3-10</sub> cycloalkyl, aryl and heteroaryl, under neutral or basic conditions. In step (e), the methylthio group in the 2-position is oxidized to the corresponding sulfone by using oxidizing agents such as chloroperoxybenzoic acid in chloroform or hydrogen peroxide in acetic acid. The methylsulfonyl group is easily exchanged in step (f) by reaction with a nucleophile having the general formula (VI) above. Since this nucleophile has an heterocyclic ring containing at least two nitrogen atoms, the second nitrogen atom of said heterocyclic ring can be acylated or sulfonylated in step (f) by treatment with an appropriate acid chloride or sulfonyl chloride R<sub>1</sub>Cl in a aprotic solvent such as dimethylformamide, pyridine or dichloromethane and, if necessary, in the presence of a base such as a tertiary amine (e.g. triethylamine). In step (g), acidic or basic hydrolysis of the amino group at position 4 of the pteridine ring is performed and results in the corresponding 4-oxopteridine derivative. In step (h), the hydroxyl group of the tautomeric form of the latter is activated by nucleophilic displacement, e.g. by preparing the 4-[(1,2,4)-triazolyl] pteridine

derivative. Finally in a first part of step (i), a nucleophilic displacement is performed by mixing the said 4-triazolylpteridine derivative with a nucleophile having the general formula (VI) above. Since this nucleophile has a heterocyclic ring containing at least two nitrogen atoms, the second nitrogen atom of each heterocyclic ring can be acylated or sulfonylated in a last part of step (i) by treating the intermediate with an appropriate acid chloride or sulfonyl chloride  $R_1Cl$  in a aprotic solvent such as dimethylformamide, pyridine or dichloromethane and, if necessary, in the presence of a base such as a tertiary amine (e.g. triethylamine).

Figure 8 schematically shows a method for making trisubstituted (wherein  $R_2$  or  $R_3$  is hydrogen) and tetrasubstituted pteridine derivatives having the formula (I) wherein  $R_4$  and  $R_5$  are identical groups having the formula (II). In step (a), a nitro group is introduced in position 5 of a 6-amino-2,4-dioxypyrimidine under strongly acidic conditions (e.g.  $HNO_3$ ,  $H_2SO_4$ ). Then, in step (b), both hydroxyl groups from the tautomeric form are converted to chloro groups by treatment with a chlorinating agent such as  $POCl_3$  or  $SOCl_2$ . Both chloro groups are then displaced in step (c) with a nucleophile having the general formula (VI), thus yielding novel 6-aminopyrimidines having the general formula (IV) wherein  $R_1$  is hydrogen and  $R_6$  is nitro. The nitro group of the latter is then reduced in step (d) to an amino group by treatment with a reducing agent (e.g.  $Pt/H_2$ ), thus yielding novel 6-aminopyrimidines having the general formula (IV) wherein  $R_1$  is hydrogen and  $R_6$  is amino. Finally, reaction of the latter with an  $\alpha$ -ketoaldoxime bearing the group  $R_2$ , wherein  $R_2$  may be *inter alia* aryl,  $C_{1-7}$  alkyl,  $C_{1-10}$  cycloalkyl or heteroaryl, regioselectively yields the desired 2,4,6-trisubstituted pteridine derivative in step (e). Alternatively, reaction of the 2,4-substituted-5,6-diaminopyrimidine from step (d) with a monosubstituted glyoxal bearing a group  $R_3$  wherein  $R_3$  may be *inter alia*  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl or heteroaryl yields the desired 2,4,7-trisubstituted pteridine derivative in step (f). Alternatively, reaction of the 2,4-substituted-5,6-diaminopyrimidine from step (d) with a disubstituted glyoxal bearing groups  $R_2$  and  $R_3$ , wherein each of  $R_2$  and  $R_3$  is independently selected (i.e.  $R_2$  and  $R_3$  may be identical or different) from the group consisting of  $C_{1-7}$  alkyl,  $C_{3-10}$  cycloalkyl, aryl and heteroaryl, under

neutral or basic conditions, yields the desired 2,4,6,7-tetrasubstituted pteridine derivative in step (g). Finally, since the nucleophile used in step (c) has a heterocyclic ring containing at least two nitrogen atoms, the second nitrogen atom can be acylated or sulfonylated in a last step (not shown in the figure) by treatment with an appropriate acid chloride or sulfonyl chloride  $R_1Cl$  in a 5 aprotic solvent such as dimethylformamide, pyridine or dichloromethane and, if necessary, in the presence of a base such as a tertiary amine (e.g. triethylamine).

In another particular embodiment, the invention relates to a group of 10 pteridine derivatives, as well as pharmaceutical compositions comprising such pteridine derivatives as active principle, having the above general formula (I) and being in the form of a pharmaceutically acceptable salt. The latter include any therapeutically active non-toxic addition salt which compounds having the 15 general formula (I) are able to form with a salt-forming agent. Such addition salts may conveniently be obtained by treating the pteridine derivatives of the invention with an appropriate salt-forming acid or base. For instance, pteridine derivatives having basic properties may be converted into the corresponding therapeutically active, non-toxic acid addition salt form by treating the free 20 base form with a suitable amount of an appropriate acid following conventional procedures. Examples of such appropriate salt-forming acids include, for instance, inorganic acids resulting in forming salts such as but not limited to hydrohalides (e.g. hydrochloride and hydrobromide), sulfate, nitrate, phosphate, diphosphate, carbonate, bicarbonate, and the like; and organic monocarboxylic or dicarboxylic acids resulting in forming salts such as, for 25 example, acetate, propanoate, hydroxyacetate, 2-hydroxypropanoate, 2-oxopropanoate, lactate, pyruvate, oxalate, malonate, succinate, maleate, fumarate, malate, tartrate, citrate, methanesulfonate, ethanesulfonate, benzoate, 2-hydroxybenzoate, 4-amino-2-hydroxybenzoate, benzene-sulfonate, p-toluenesulfonate, salicylate, p-aminosalicylate, pamoate, 30 bitartrate, camphorsulfonate, edetate, 1,2-ethanedisulfonate, fumarate, glucoheptonate, gluconate, glutamate, hexylresorcinate, hydroxynaphtoate, hydroxyethanesulfonate, mandelate, methylsulfate, pantothenate, stearate, as well as salts derived from ethanedioic, propanedioic, butanedioic, (Z)-2-



butenedioic, (E)-butenedioic, 2-hydroxybutanedioic, 2,3-dihydroxybutanedioic, 2-hydroxy-1,2,3-propanetricarboxylic and cyclohexanesulfamic acids and the like.

Pteridine derivatives (I) having acidic properties may be converted in a similar manner into the corresponding therapeutically active, non-toxic base addition salt form. Examples of appropriate salt-forming bases include, for instance, inorganic bases like metallic hydroxides such as but not limited to those of alkali and alkaline-earth metals like calcium, lithium, magnesium, potassium and sodium, or zinc, resulting in the corresponding metal salt; organic bases such as but not limited to ammonia, alkylamines, benzathine, hydrabamine, arginine, lysine, N,N'-dibenzylethylenediamine, chlorprocaine, choline, diethanolamine, ethylene-diamine, N-methylglucamine, procaine and the like.

Reaction conditions for treating the pteridine derivatives (I) of this invention with an appropriate salt-forming acid or base are similar to standard conditions involving the same acid or base but different organic compounds with basic or acidic properties, respectively. Preferably, in view of its use in a pharmaceutical composition or in the manufacture of medicament for treating specific diseases, the pharmaceutically acceptable salt will be designed, i.e. the salt-forming acid or base will be selected so as to impart greater water-solubility, lower toxicity, greater stability and/or slower dissolution rate to the pteridine derivative of this invention.

The present invention further provides the use of a pteridine derivative represented by the general formula (I), or a pharmaceutically acceptable salt or a solvate thereof, as a biologically-active ingredient, i.e. an active principle, especially as a medicine or a diagnostic agent or for the manufacture of a medicament or a diagnostic kit. In particular the said medicament may be for the prevention or treatment of a pathologic condition selected from the group consisting of:

- immune disorders, in particular organ and cells transplant rejections, and autoimmune disorders,
- cardiovascular disorders,
- allergic conditions,

- disorders of the central nervous system,
- TNF- $\alpha$ -related disorders, and
- cell proliferative disorders.

The pathologic conditions and disorders concerned by the said use, and  
 5 the corresponding methods of prevention or treatment, are detailed  
 hereinbelow. Any of the uses mentioned with respect to the present invention  
 may be restricted to a non-medical use (e.g. in a cosmetic composition), a  
 non-therapeutic use, a non-diagnostic use, a non-human use (e.g. in a  
 veterinary composition), or exclusively an *in-vitro* use, or a use with cells  
 10 remote from an animal.

The invention further relates to a pharmaceutical composition comprising:

- (a) one or more pteridine derivatives represented by the general formula (I),  
 and
- (b) one or more pharmaceutically acceptable carriers.

15 In a third embodiment, this invention provides combinations, preferably  
 synergistic combinations, of one or more pteridine derivative represented by  
 the general formula (I) with one or more biologically-active drugs being  
 preferably selected from the group consisting of immunosuppressant and/or  
 immunomodulator drugs, antineoplastic drugs, anti-histamines, inhibitors of  
 20 allergy-causative agents (anti-allergic drugs) and antiviral agents. As is  
 conventional in the art, the evaluation of a synergistic effect in a drug  
 combination may be made by analyzing the quantification of the interactions  
 between individual drugs, using the median effect principle described by Chou  
 et al. in *Adv. Enzyme Reg.* (1984) 22:27. Briefly, this principle states that  
 25 interactions (synergism, additivity, antagonism) between two drugs can be  
 quantified using the combination index (hereinafter referred as CI) defined by  
 the following equation:

$$CI_x = \frac{ED_x^{1c}}{ED_x^{1a}} + \frac{ED_x^{2c}}{ED_x^{2a}}$$

wherein  $ED_x$  is the dose of the first or respectively second drug used alone  
 30 (1a, 2a), or in combination with the second or respectively first drug (1c, 2c),  
 which is needed to produce a given effect. The said first and second drug

have synergistic or additive or antagonistic effects depending upon  $CI < 1$ ,  $CI = 1$ , or  $CI > 1$ , respectively. As will be explained in more detail herein-below, this principle may be applied to a number of desirable effects such as, but not limited to, an activity against transplant rejection, an activity against immunosuppression or immunomodulation, an activity against allergy or an activity against cell proliferation.

For instance the present invention relates to a pharmaceutical composition or combined preparation having synergistic effects against immunosuppression or immunomodulation and containing:

- 10 (a) one or more immunosuppressant and/or immunomodulator drugs, and
- (b) at least one pteridine derivative represented by the general formula (I), and
- (c) optionally one or more pharmaceutical excipients or pharmaceutically acceptable carriers,
- 15 for simultaneous, separate or sequential use in the treatment or prevention of autoimmune disorders and/or in transplant-rejections.

Suitable immunosuppressant drugs for inclusion in the synergistic compositions or combined preparations of this invention belong to a well known therapeutic class. They are preferably selected from the group consisting of cyclosporin A, substituted xanthines (e.g. methylxanthines such as pentoxifylline), daltroban, sirolimus, tacrolimus, rapamycin (and derivatives thereof such as defined below), leflunomide (or its main active metabolite A771726, or analogs thereof called malononitrilamides), mycophenolic acid and salts thereof (including the sodium salt marketed under the trade name Mofetil<sup>®</sup>), adrenocortical steroids, azathioprine, brequinar, gusperimus, 6-mercaptapurine, mizoribine, chloroquine, hydroxychloroquine and monoclonal antibodies with immunosuppressive properties (e.g. etanercept, infliximab or kineret). Adrenocortical steroids within the meaning of this invention mainly include glucocorticoids such as but not limited to ciprocinonide, desoxycorticosterone, fludrocortisone, flumoxonide, hydrocortisone, naflocort, procinonide, timobesone, tipredane, dexamethasone, methylprednisolone, methotrexate, prednisone, prednisolone, triamcinolone and pharmaceutically acceptable salts thereof. Rapamycin derivatives as referred herein include O-

alkylated derivatives, particularly 9-deoxorapamycins, 26-dihydrorapamycins, 40-O-substituted rapamycins and 28,40-O,O-disubstituted rapamycins (as disclosed in U.S. Patent No. 5,665,772) such as 40-O-(2-hydroxy) ethyl rapamycin – also known as SDZ-RAD -, pegylated rapamycin (as disclosed in  
5 U.S. Patent No. 5,780,462), ethers of 7-desmethylrapamycin (as disclosed in U.S. Patent No. 6,440,991) and polyethylene glycol esters of SDZ-RAD (as disclosed in U.S. Patent No. 6,331,547).

Suitable immunomodulator drugs for inclusion into the synergistic immunomodulating pharmaceutical compositions or combined preparations of  
10 this invention are preferably selected from the group consisting of acemannan, amiprilose, bucillamine, dimepranol, ditiocarb sodium, imiquimod, Inosine Pranobex, interferon- $\beta$ , interferon- $\gamma$ , lentinan, levamisole, lisophylline, pidotimod, romurtide, platonin, procodazole, propagermanium, thymomodulin, thymopentin and ubenimex.

15 Synergistic activity of the pharmaceutical compositions or combined preparations of this invention against immunosuppression or immuno-modulation may be readily determined by means of one or more lymphocyte activation tests. Usually activation is measured via lymphocyte proliferation. Inhibition of proliferation thus always means immunosuppression under the  
20 experimental conditions applied. There exist different stimuli for lymphocyte activation, in particular:

- a) co-culture of lymphocytes of different species (mixed lymphocyte reaction, hereinafter referred as MLR) in a so-called mixed lymphocyte culture test: lymphocytes expressing different minor and major antigens of the HLA-DR type (= alloantigens) activate each other non-specifically;  
25
- b) a CD3 assay wherein there is an activation of the T-lymphocytes via an exogenously added antibody (OKT3). This antibody reacts against a CD3 molecule located on the lymphocyte membrane which has a co-stimulatory function. Interaction between OKT3 and CD3 results in T-cell activation  
30 which proceeds via the  $\text{Ca}^{2+}$ /calmodulin/calcineurin system and can be inhibited e.g. by cyclosporin A (hereinafter referred as CyA);

c) a CD28 assay wherein specific activation of the T-lymphocyte proceeds via an exogenously added antibody against a CD28 molecule which is also located on the lymphocyte membrane and delivers strong co-stimulatory signals. This activation is  $\text{Ca}^{2+}$ -independent and thus cannot be inhibited by CyA.

Determination of the immunosuppressing or immunomodulating activity of the pteridine derivatives of this invention, as well as synergistic combinations comprising them, is preferably based on the determination of one or more, preferably at least three lymphocyte activation *in vitro* tests, more preferably including at least one of the MLR test, CD3 assay and CD28 assay referred above. Preferably the lymphocyte activation *in vitro* tests used include at least two assays for two different clusters of differentiation preferably belonging to the same general type of such clusters and more preferably belonging to type I transmembrane proteins. Optionally the determination of the immunosuppressing or immunomodulating activity may be performed on the basis of other lymphocyte activation *in vitro* tests, for instance by performing a TNF- $\alpha$  assay or an IL-1 assay or an IL-6 assay or an IL-10 assay or an IL-12 assay or an assay for a cluster of differentiation belonging to a further general type of such clusters and more preferably belonging to type II transmembrane proteins such as, but not limited to, CD69, CD 71 or CD134.

The synergistic effect may be evaluated by the median effect analysis method described herein-before. Such tests may for instance, according to standard practice in the art, involve the use of equipment, such as flow cytometer, being able to separate and sort a number of cell subcategories at the end of the analysis, before these purified batches can be analysed further.

Synergistic activity of the pharmaceutical compositions of this invention in the prevention or treatment of transplant rejection may be readily determined by means of one or more leukocyte activation tests performed in a Whole Blood Assay (hereinafter referred as WBA) described for instance by Lin et al. in *Transplantation* (1997) 63:1734-1738. WBA used herein is a lymphoproliferation assay performed *in vitro* using lymphocytes present in the whole blood, taken from animals that were previously given the pteridine derivative, and optionally the other immunosuppressant drug, *in vivo* (more

details are given in example 118). Hence this assay reflects the *in vivo* effect of substances as assessed by an *in vitro* read-out assay. The synergistic effect may be evaluated by the median effect analysis method described herein-before. Various organ transplantation models in animals are also  
5 available *in vivo*, which are strongly influenced by different immunogenicities, depending on the donor and recipient species used and depending on the nature of the transplanted organ. The survival time of transplanted organs can thus be used to measure the suppression of the immune response.

The pharmaceutical composition or combined preparation with synergistic  
10 activity against immunosuppression or immunomodulation according to this invention may contain the pteridine derivative of formula (I) over a broad content range depending on the contemplated use and the expected effect of the preparation. Generally, the pteridine derivative content of the combined preparation is within the range of 0.1 to 99.9% by weight, preferably from 1 to  
15 99% by weight, more preferably from 5 to 95% by weight.

The invention further relates to a composition or combined preparation having synergistic effects against cell proliferation and containing:

- (a) one or more antineoplastic drugs, and
- (b) at least one pteridine derivative represented by the general formula (I), and  
20 (c) optionally one or more pharmaceutical excipients or pharmaceutically acceptable carriers,

for simultaneous, separate or sequential use in the treatment or prevention of cell proliferative disorders.

Suitable antineoplastic drugs for inclusion into the synergistic  
25 antiproliferative pharmaceutical compositions or combined preparations of this invention are preferably selected from the group consisting of alkaloids, alkylating agents (including but not limited to alkyl sulfonates, aziridines, ethylenimines, methylmelamines, nitrogen mustards and nitrosoureas), antibiotics, antimetabolites (including but not limited to folic acid analogs,  
30 purine analogs and pyrimidine analogs), enzymes, interferon and platinum complexes. More specific examples include acivicin; aclarubicin; acodazole; acronine; adozelesin; aldesleukin; altretamine; ambomycin; ametantrone;

aminogluthethimide; amsacrine; anastrozole; anthramycin; asparaginase;  
 asperlin; azacitidine; azetepa; azotomycin; batimastat; benzodepa;  
 bicalutamide; bisantrene; bisnafide; bizelesin; bleomycin; brequinar;  
 bropirimine; busulfan; cactinomycin; calusterone; caracemide; carbetimer;  
 5 carboplatin; carmustine; carubicin; carzelesin; cedefingol; chlorambucil;  
 cirolemycin; cisplatin; cladribine; crisnatol; cyclophosphamide; cytarabine;  
 dacarbazine; dactinomycin; daunorubicin; decitabine; dexormaplatin;  
 dezaguanine; diaziquone; docetaxel; doxorubicin; droloxifene;  
 dromostanolone; duazomycin; edatrexate; eflomithine; elsamitrucin;  
 10 enloplatin; enpromate; epipropidine; epirubicin; erbulozole; esorubicin;  
 estramustine; etanidazole; ethiodized oil I 131; etoposide; etoprine; fadrozole;  
 fazarabine; fenretinide; floxuridine; fludarabine; fluorouracil; flurocitabine;  
 fosquidone; fostriecin; gemcitabine; Gold 198; hydroxyurea; idarubicin;  
 ifosfamide; ilmofofosine; interferon  $\alpha$ -2a; interferon  $\alpha$ -2b; interferon  $\alpha$ -n1;  
 15 interferon  $\alpha$ -n3; interferon  $\beta$ -1a; interferon  $\gamma$ -1b; iproplatin; irinotecan;  
 lanreotide; letrozole; leuprolide; liarozole; lometrexol; lomustine; losoxantrone;  
 masoprocol; maytansine; mechlorethamine; megestrol; melengestrol;  
 melphalan; menogaril; mercaptopurine; methotrexate; metoprine; meturedopa;  
 mitindomide; mitocarcin; mitocromin; mitogillin; mitomalcin; mitomycin;  
 20 mitosper; mitotane; mitoxantrone; mycophenolic acid; nocodazole; nogala-  
 mycin; ormaplatin; oxisuran; paclitaxel; pegaspargase; peliomycin;  
 pentamustine; peplomycin; perfosfamide; pipobroman; piposulfan;  
 piroxantrone; plicamycin; plomestane; porfimer; porfiromycin; prednimustine;  
 procarbazine; puromycin; pyrazofurin; riboprine; rogletimide; safingol;  
 25 semustine; simtrazene; sparfosate; sparsomycin; spirogermanium;  
 spiromustine; spiroplatin; streptonigrin; streptozocin; strontium 89 chloride;  
 sulofenur; talisomycin; taxane; taxoid; tecogalan; tegafur; teloxantrone;  
 temoporfin; teniposide; teroxirone; testolactone; thiamiprine; thioguanine;  
 thiotepa; tiazofurin; tirapazamine; topotecan; toremifene; trestolone; tricirbine;  
 30 trimetrexate; triptorelin; tubulozole; uracil mustard; uredepa; vapreotide;  
 verteporfin; vinblastine; vincristine; vindesine; vinepidine; vinglycinate;  
 vinleurosine; vinorelbine; vinrosidine; vinzolidine; vorozole; zeniplatin;  
 zinostatin; zorubicin; and their pharmaceutically acceptable salts.

Other suitable anti-neoplastic compounds include 20-epi-1,25 dihydroxyvitamin D<sub>3</sub>; 5-ethynyluracil; abiraterone; aclarubicin; acylfulvene; adecypenol; adozelesin; aldesleukin; ALL-TK antagonists; altretamine; ambamustine; amidox; amifostine; aminolevulinic acid; amrubicin; amsacrine; 5 anagrelide; anastrozole; andrographolide; angiogenesis inhibitors; antagonist D; antagonist G; antarelix; anti-dorsalizing morphogenetic protein-1; anti-androgens such as, but not limited to, benorterone, cioteronel, cyproterone, delmadinone, oxendolone, topterone, zanoterone and their pharmaceutically acceptable salts; anti-estrogens such as, but not limited to, clometherone; 10 delmadinone; nafoxidine; nitromifene; raloxifene; tamoxifen; toremifene; trioxifene and their pharmaceutically acceptable salts; antineoplaston; antisense oligonucleotides; aphidicolin glycinate; apoptosis gene modulators; apoptosis regulators; apurinic acid; ara-CDP-DL-PTBA; arginine deaminase; asulacrine; atamestane; atrimustine; axinastatin; azasetron; azatoxin; 15 azatyrosine; baccatin III derivatives; balanol; batimastat; BCR/ABL antagonists; benzochlorins; benzoylstauroporine;  $\beta$ -lactam derivatives;  $\beta$ -alethine; betaclamycin B; betulinic acid; bFGF inhibitor; bicalutamide; bisantrene; bisaziridinylspermine; bisnafide; bistratene A; bizelesin; breflate; bropirimine; budotitane; buthionine sulfoximine; calcipotriol; calphostin C; 20 camptothecin derivatives; canarypox IL-2; capecitabine; carboxamide-amino-triazole; carboxyamidotriazole; CaRest M3; CARN 700; cartilage derived inhibitor; carzelesin; casein kinase inhibitors; castanospermine; cecropin B; cetorelix; chlorins; chloroquinoxaline sulfonamide; cicaprost; cis-porphyrin; clomifene and analogues thereof; clotrimazole; collismycin A and B; 25 combretastatin and analogues thereof; conagenin; crambescidin 816; cryptophycin and derivatives thereof; curacin A; cyclopentantraquinones; cycloplatam; cypemycin; cytarabine; cytolytic factor; cytostatin; dacliximab; dehydroididemnin B; deslorelin; dexifosfamide; dexrazoxane; dexverapamil; didemnin B; didox; diethylnorspermine; dihydro-5-azacytidine; dihydrotaxol; 30 dioxamycin; diphenyl spiromustine; docosanol; dolasetron; doxifluridine; droloxifene; dronabinol; duocarmycin SA; ebselen; ecomustine; edelfosine; edrecolomab; elemene; emitefur; epristeride; estrogen agonists and antagonists; exemestane; filgrastim; finasteride; flavopiridol; flezelastine;



fluasterone; fluorodaunorubicin; forfenimex; formestane; fotemustine;  
 gadolinium texaphyrin; gallium nitrate; galocitabine; ganirelix; gelatinase  
 inhibitors; glutathione inhibitors; hepsulfam; heregulin; hexamethylene  
 bisacetamide; hypericin; ibandronic acid; idoxifene; idramantone; ilomastat;  
 5 imidazoacridones; imiquimod; immunostimulant peptides; insulin-like growth  
 factor-1 receptor inhibitor; interferon agonists; iobenguane; iododoxorubicin;  
 ipomeanol; irinotecan; iroplact; irsogladine; isobengazole; isohomohalicondrin  
 B; itasetron; jasplakinolide; kahalalide F; lamellarin-N; leinamycin;  
 lenograstim; lentinan; leptolstatin; leukemia inhibiting factor; leuprorelin;  
 10 levamisole; liarozole; lissoclinamide; lobaplatin; lombricine; lonidamine;  
 lovastatin; loxoribine; lurtotecan; lutetium texaphyrin; lysofylline; mannostatin  
 A; marimastat; masoprocil; maspin; matrilysin inhibitors; matrix  
 metalloproteinase inhibitors; merbarone; meterelin; methioninase;  
 metoclopramide; MIF inhibitors; mifepristone; miltefosine; mirimostim;  
 15 mitoguazone; mitolactol; mitonafide; mitotoxin fibroblast growth factor-saporin;  
 mofarotene; molgramostim; human chorionic gonadotrophin monoclonal  
 antibody; mopidamol; mycaperoxide B; myriaporone; N-acetyldinaline; N-  
 substituted benzamides; nafarelin; nagrestip; naloxone; pentazocine; napavin;  
 naphterpin; nartograstim; nedaplatin; nemorubicin; neridronic acid; neutral  
 20 endopeptidase; nilutamide; nisamycin; nitric oxide modulators; nitroxide  
 antioxidant; nitrullyn; octreotide; okicenone; onapristone; ondansetron;  
 ondansetron; oracin; osaterone; oxaliplatin; oxanunomycin; palauamine;  
 palmitoylrhizoxin; pamidronic acid; panaxytriol; panomifene; parabactin;  
 pazelliptine; peldesine; pentosan; pentostatin; pentozole; perflubron; perillyl  
 25 alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil;  
 pilocarpine; pirarubicin; piritrexim; placetin A and B; plasminogen activator  
 inhibitor; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein  
 kinase C inhibitors; protein tyrosine phosphatase inhibitors; purine nucleoside  
 phosphorylase inhibitors; purpurins; pyrazoloacridine; raltitrexed; ramosetron;  
 30 ras farnesyl protein transferase inhibitors; ras inhibitors; ras-GAP inhibitors;  
 retelliptine; rhenium 186 etidronate; rhizoxin; retinamide; rohitukine; romurtide;  
 roquinimex; rubiginone B1; ruboxyl; saintopin; sarcophytol A; sargramostim;  
 sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol;

somatomedin binding protein; sonermin; sparfosic acid; spicamycin D; splenopentin; spongistatin 1; squalamine; stem-cell division inhibitors; stipiamide; stromelysin inhibitors; sulfinosine; suradista; suramin; swainsonine; tallimustine; tamoxifen; tauromustine; tazarotene; tecogalan; 5 tellurapyrylium; telomerase inhibitors; temozolomide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thiocoraline; thrombopoietin; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone; tin ethyl etiopurpurin; titanocene; topsentin; tretinoin; triacetyluridine; tropisetron; turosteride; tyrosine kinase inhibitors; tyrphostins; ubenimex; urogenital sinus- 10 derived growth inhibitory factor; urokinase receptor antagonists; variolin B; velaresol; veramine; verdins; verteporfin; vinxaltine; vitaxin; zanoterone; zilascorb; and their pharmaceutically acceptable salts.

Synergistic activity of the pharmaceutical compositions or combined preparations of this invention against cell proliferation may be readily 15 determined by means of one or more tests such as, but not limited to, the measurement of the radioactivity resulting from the incorporation of <sup>3</sup>H-thymidine in culture of tumor cell lines. For instance, different tumor cell lines are selected in order to evaluate the anti-tumor effects of the test compounds, such as but not limited to:

- 20     - RPMI1788: human Peripheral Blood Leucocytes (PBL) Caucasian tumor line,
- Jurkat: human acute T cell leukemia,
- EL4: C57Bl/6 mouse lymphoma, or
- THP-1: human monocyte tumor line.
- 25     Depending on the selected tumor cell line, different culture media may be used, such as for example:
  - for RPMI1788 and THP-1: RPMI-1640 + 10% FCS + 1% NEAA + 1% sodium pyruvate + 5x10<sup>-5</sup> mercapto-ethanol + antibiotics (G-418 0.45 µg/ml).
  - 30     - for Jurkat and EL4: RPMI-1640 + 10% FCS + antibiotics (G-418 0.45 µg/ml).

In a specific embodiment of the synergy determination test, the tumor cell lines are harvested and a suspension of  $0.27 \times 10^6$  cells/ml in whole medium is prepared. The suspensions (150  $\mu$ l) are added to a microtiter plate in triplicate. Either complete medium (controls) or the test compounds at the test concentrations (50  $\mu$ l) are added to the cell suspension in the microtiter plate. The cells are incubated at 37°C under 5% CO<sub>2</sub> for about 16 hours. <sup>3</sup>H-thymidine is added, and the cells incubated for another 8 hours. The cells are harvested and radioactivity is measured in counts per minute (CPM) in a  $\beta$ -counter. The <sup>3</sup>H-thymidine cell content, and thus the measured radioactivity, is proportional to the proliferation of the cell lines. The synergistic effect is evaluated by the median effect analysis method as disclosed herein-before.

The pharmaceutical composition or combined preparation with synergistic activity against cell proliferation according to this invention may contain the pteridine derivative of formula (I) over a broad content range depending on the contemplated use and the expected effect of the preparation. Generally, the pteridine derivative content of the combined preparation is within the range of 0.1 to 99.9% by weight, preferably from 1 to 99% by weight, more preferably from 5 to 95% by weight.

The invention further relates to a pharmaceutical composition or combined preparation having synergistic effects against a viral infection and containing:

- (a) one or more anti-viral agents, and
- (b) at least one pteridine derivative represented by the general formula (I), and
- (c) optionally one or more pharmaceutical excipients or pharmaceutically acceptable carriers,

for simultaneous, separate or sequential use in the treatment or prevention of a viral infection.

Suitable anti-viral agents for inclusion into the synergistic antiviral compositions or combined preparations of this invention include, for instance, retroviral enzyme inhibitors belonging to categories well known in the art, such as HIV-1 IN inhibitors, nucleoside reverse transcriptase inhibitors (e.g. zidovudine, lamivudine, didanosine, stavudine, zalcitabine and the like), non-nucleoside reverse transcriptase inhibitors (e.g. nevirapine, delavirdine and the like), other reverse transcriptase inhibitors (e.g. foscarnet sodium and the

like), and HIV-1 protease inhibitors (e.g. saquinavir, ritonavir, indinavir, nelfinavir and the like). Other suitable antiviral agents include for instance acemannan, acyclovir, adefovir, alovudine, alvircept, amantadine, aranotin, arildone, atevirdine, avridine, cidofovir, cipamfylline, cytarabine, desciclovir, 5 disoxaril, edoxudine, envirodene, enviroxime, famciclovir, famotone, fiacitabine, fialuridine, floxuridine, fosarilate, fosfonet, ganciclovir, idoxuridine, kethoxal, lobucavir, memotone, methisazone, penciclovir, pirodavir, somantadine, sorivudine, tilorone, trifluridine, valaciclovir, vidarabine, viroxime, zinviroxime, moroxydine, podophyllotoxin, ribavirine, rimantadine, 10 stallimycine, statolon, tromantadine and xenazoic acid, and their pharmaceutically acceptable salts.

Especially relevant to this aspect of the invention is the inhibition of the replication of viruses selected from the group consisting of picorna-, toga-, bunya, orthomyxo-, paramyxo-, rhabdo-, retro-, arena-, hepatitis B-, hepatitis 15 C-, hepatitis D-, adeno-, vaccinia-, papilloma-, herpes-, corona-, varicella- and zoster-virus, in particular human immunodeficiency virus (HIV). Synergistic activity of the pharmaceutical compositions or combined preparations of this invention against viral infection may be readily determined by means of one or more tests such as, but not limited to, the isobologram method, as previously 20 described by Elion et al. in *J. Biol. Chem.* (1954) 208:477-488 and by Baba et al. in *Antimicrob. Agents Chemother.* (1984) 25:515-517, using  $EC_{50}$  for calculating the fractional inhibitory concentration (hereinafter referred as FIC). When the minimum FIC index corresponding to the FIC of combined compounds (e.g.,  $FIC_x + FIC_y$ ) is equal to 1.0, the combination is said to be 25 additive; when it is between 1.0 and 0.5, the combination is defined as subsynergistic, and when it is lower than 0.5, the combination is by defined as synergistic. When the minimum FIC index is between 1.0 and 2.0, the combination is defined as subantagonistic and, when it is higher than 2.0, the combination is defined as antagonistic.

30 The pharmaceutical composition or combined preparation with synergistic activity against viral infection according to this invention may contain the pteridine derivative of formula (I) over a broad content range depending on the contemplated use and the expected effect of the preparation. Generally, the

pteridine derivative content of the combined preparation is within the range of 0.1 to 99.9% by weight, preferably from 1 to 99% by weight, more preferably from 5 to 95% by weight.

5 The pharmaceutical compositions and combined preparations according to this invention may be administered orally or in any other suitable fashion. Oral administration is preferred and the preparation may have the form of a tablet, aqueous dispersion, dispersable powder or granule, emulsion, hard or soft capsule, syrup, elixir or gel. The dosing forms may be prepared using any method known in the art for manufacturing these  
10 pharmaceutical compositions and may comprise as additives sweeteners, flavoring agents, coloring agents, preservatives and the like. Carrier materials and excipients are detailed hereinbelow and may include, *inter alia*, calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, binding agents and the like.  
15 The pharmaceutical composition or combined preparation of this invention may be included in a gelatin capsule mixed with any inert solid diluent or carrier material, or has the form of a soft gelatin capsule, in which the ingredient is mixed with a water or oil medium. Aqueous dispersions may comprise the biologically active composition or combined preparation in  
20 combination with a suspending agent, dispersing agent or wetting agent. Oil dispersions may comprise suspending agents such as a vegetable oil. Rectal administration is also applicable, for instance in the form of suppositories or gels. Injection (e.g. intramuscularly or intraperitoneously) is also applicable as a mode of administration, for instance in the form of injectable solutions or  
25 dispersions, depending upon the disorder to be treated and the condition of the patient.

Auto-immune disorders to be prevented or treated by the pharmaceutical compositions or combined preparations of this invention include both systemic auto-immune diseases such as but not limited to lupus  
30 erythematosus, psoriasis, vasculitis, polymyositis, scleroderma, multiple sclerosis, ankylosing spondylitis, rheumatoid arthritis and Sjögren syndrome; auto-immune endocrine disorders such as thyroiditis; and organ-specific auto-immune diseases such as but not limited to Addison disease, hemolytic or

pernicious anemia, Goodpasture syndrome, Graves disease, idiopathic thrombocytopenic purpura, insulin-dependent diabetes mellitus, juvenile diabetes, uveitis, Crohn's disease, ulcerative colitis, pemphigus, atopic dermatitis, autoimmune hepatitis, primary biliary cirrhosis, autoimmune  
5 pneumonitis, autoimmune carditis, myasthenia gravis, glomerulonephritis and spontaneous infertility.

Transplant rejections to be prevented or treated by the pharmaceutical compositions or combined preparations of this invention include the rejection of transplanted or grafted organs or cells (both allografts and xenografts),  
10 such as but not limited to host versus graft reaction disease. The term "organ" as used herein means all organs or parts of organs in mammals, in particular humans, such as but not limited to kidney, lung, bone marrow, hair, cornea, eye (vitreous), heart, heart valve, liver, pancreas, blood vessel, skin, muscle, bone, intestine or stomach. "Rejection" as used herein mean all reactions of  
15 the recipient body or of the transplanted organ which in the end lead to cell or tissue death in the transplanted organ or adversely affect the functional ability and viability of the transplanted organ or the recipient. In particular, this means acute and chronic rejection reactions. Also included in this invention is preventing or treating the rejection of cell transplants and xenotransplantation.  
20 The major hurdle for xenotransplantation is that even before the T lymphocytes, responsible for the rejection of allografts, are activated, the innate immune system, especially T-independent B lymphocytes and macrophages are activated. This provokes two types of severe and early acute rejection called hyper-acute rejection and vascular rejection,  
25 respectively. The present invention addresses the problem that conventional immunosuppressant drugs like cyclosporin A are ineffective in xenotransplantation. The ability of the compounds of this invention to suppress T-independent xeno-antibody production as well as macrophage activation may be evaluated in the ability to prevent xenograft rejection in athymic, T-deficient  
30 mice receiving xenogenic hamster-heart grafts.

Cell proliferative disorders to be prevented or treated by the pharmaceutical compositions or combined preparations of this invention include any kind of tumor progression or invasion or metastasis inhibition of a

cancer, preferably one selected from the group consisting of lung cancer, leukaemia, ovarian cancer, sarcoma, Kaposi's sarcoma, meningioma, colon cancer, lymph node tumor, glioblastoma multiforme, prostate cancer or skin carcinoma.

5 CNS disorders to be prevented or treated by the pharmaceutical compositions of this invention include cognitive pathologies such as dementia, cerebral ischemia, trauma, epilepsy, schizophrenia, chronic pain and neurologic disorders such as but not limited to depression, social phobia and obsessive compulsive disorders.

10 Cardiovascular disorders to be prevented or treated by the pharmaceutical compositions of this invention include ischemic disorders, infarct or reperfusion damage, atherosclerosis and stroke.

Allergic conditions to be prevented or treated by the pharmaceutical compositions of this invention include those caused by the pollen of gramineae, 15 the presence of pets, as well as more severe forms, such as asthma, characterized by inflammation of airways and bronchospasm. Without wishing to be bound by theory, the antiallergic effect of the compounds of the invention may be related to their suppression of certain B-cell activation pathways, which can lead to the suppression of IgE release. It may also be related to 20 their properties of inhibiting certain Th2 cytokines, such as IL-5, IL-13 or IL-10, involved in asthma.

TNF- $\alpha$ -related disorders to be prevented or treated by the pharmaceutical compositions of this invention include the following:

- septic or endotoxic shock or sepsis, especially in patients with a serum 25 level of interleukin-6 above 1,000 pg/ml at start of treatment;
- vascular TNF- $\alpha$ - mediated diseases such as, but not limited to, disseminated intravascular coagulation and Kawasaki's pathology;
- pathologies and conditions associated with and/or induced by abnormal levels of TNF- $\alpha$  (herein defined as exceeding by at least 10 % and at 30 most 500% the TNF- $\alpha$  level present in a normal healthy subject) occurring in a systemic, localized or particular tissue type or location in the body of the mammal; such tissue types include, but are not limited to, blood, lymph, liver, kidney, spleen, heart muscle or blood vessels,

- 5 brain or spinal cord white matter or grey matter, cartilage, ligaments, tendons, lung, pancreas, ovary, testes and prostate. Abnormal TNF levels can also be localized to specific regions or cells in the body, such as joints, nerve blood vessel junctions and bones. Such pathologies
- 10 include alcohol-induced hepatitis; neurodegenerative diseases such as extrapyramidal and cerebellar disorders including lesions of the corticospinal system; disorders of the basal ganglia; hyperkinetic movement disorders such as chorea; drug-induced movement disorders; hypokinetic movement disorders, such as Parkinson's disease; spinocerebellar degenerations, such as spinal ataxia, multiple systems degenerations (including Dejerine-Klumpke syndrome) and systemic disorders (including Refsum's disease, abetalipoproteinemia, ataxia and telangiectasia); disorders of the motor unit, such as neurogenic muscular atrophies (anterior horn cell degeneration, such
- 15 as amyotrophic lateral sclerosis, infantile spinal muscular atrophy and juvenile spinal muscular atrophy); Alzheimer's disease; Wernicke-Korsakoff syndrome; Creutzfeldt-Jakob disease; Hallerorden-Spatz disease; and primary or secondary myelodysplastic syndromes;
- toxic effects of TNF- $\alpha$  and/or anti-cancer chemotherapeutic agents,
  - 20 especially side effects associated with TNF generation during neoplastic therapy, for instance following use of cisplatin;
  - injuries after irradiation of a tissue of a mammal by radio-elements, such as but not limited to radiation-induced graft-versus-host disease; and
  - cachexia and similar chronic wasting diseases, whether associated with
  - 25 cancer or with other chronic diseases such as malabsorptive disorders, excessive physical stress, eating disorders and AIDS.

The medicament of this invention may be for prophylactic use, i.e. where circumstances are such that an elevation in the TNF level might be expected or alternatively, may be for use in reducing the TNF level after it has reached

30 an undesirably high level or as the TNF level is rising.

The term "pharmaceutically acceptable carrier or excipient" as used herein in relation to pharmaceutical compositions and combined preparations means any material or substance with which the active principle, i.e. the



pteridine derivative of formula (II), and optionally the immunosuppressant or immunomodulator or antineoplastic drug or antiviral agent, may be formulated in order to facilitate its application or dissemination to the locus to be treated, for instance by dissolving, dispersing or diffusing the said composition, and/or to facilitate its storage, transport or handling without impairing its effectiveness. The pharmaceutically acceptable carrier may be a solid or a liquid or a gas which has been compressed to form a liquid, i.e. the compositions of this invention can suitably be used as concentrates, emulsions, solutions, granulates, dusts, sprays, aerosols, pellets or powders.

10        Suitable pharmaceutical carriers for use in the said pharmaceutical compositions and their formulation are well known to those skilled in the art. There is no particular restriction to their selection within the present invention although, due to the usually low or very low water-solubility of the pteridine derivatives of this invention, special attention will be paid to the selection of  
15        suitable carrier combinations that can assist in properly formulating them in view of the expected time release profile. Suitable pharmaceutical carriers include additives such as wetting agents, dispersing agents, stickers, adhesives, emulsifying or surface-active agents, thickening agents, complexing agents, gelling agents, solvents, coatings, antibacterial and  
20        antifungal agents (for example phenol, sorbic acid, chlorobutanol), isotonic agents (such as sugars or sodium chloride) and the like, provided the same are consistent with pharmaceutical practice, i.e. carriers and additives which do not create permanent damage to mammals. The pharmaceutical compositions of the present invention may be prepared in any known manner,  
25        for instance by homogeneously mixing, dissolving, spray-drying, coating and/or grinding the active ingredients, in a one-step or a multi-steps procedure, with the selected carrier material and, where appropriate, the other additives such as surface-active agents. may also be prepared by micronisation, for instance in view to obtain them in the form of microspheres  
30        usually having a diameter of about 1 to 10  $\mu\text{m}$ , namely for the manufacture of microcapsules for controlled or sustained release of the biologically active ingredient(s).

Suitable surface-active agents to be used in the pharmaceutical compositions of the present invention are non-ionic, cationic and/or anionic materials having good emulsifying, dispersing and/or wetting properties. Suitable anionic surfactants include both water-soluble soaps and water-soluble synthetic surface-active agents. Suitable soaps are alkaline or alkaline-earth metal salts, unsubstituted or substituted ammonium salts of higher fatty acids ( $C_{10}$ - $C_{22}$ ), e.g. the sodium or potassium salts of oleic or stearic acid, or of natural fatty acid mixtures obtainable from coconut oil or tallow oil. Synthetic surfactants include sodium or calcium salts of polyacrylic acids; fatty sulphonates and sulphates; sulphonated benzimidazole derivatives and alkylarylsulphonates. Fatty sulphonates or sulphates are usually in the form of alkaline or alkaline-earth metal salts, unsubstituted ammonium salts or ammonium salts substituted with an alkyl or acyl radical having from 8 to 22 carbon atoms, e.g. the sodium or calcium salt of lignosulphonic acid or dodecylsulphonic acid or a mixture of fatty alcohol sulphates obtained from natural fatty acids, alkaline or alkaline-earth metal salts of sulphuric or sulphonic acid esters (such as sodium lauryl sulphate) and sulphonic acids of fatty alcohol/ethylene oxide adducts. Suitable sulphonated benzimidazole derivatives preferably contain 8 to 22 carbon atoms. Examples of alkylarylsulphonates are the sodium, calcium or alcanolamine salts of dodecylbenzene sulphonic acid or dibutyl-naphtalenesulphonic acid or a naphtalene-sulphonic acid/formaldehyde condensation product. Also suitable are the corresponding phosphates, e.g. salts of phosphoric acid ester and an adduct of p-nonylphenol with ethylene and/or propylene oxide, or phospholipids. Suitable phospholipids for this purpose are the natural (originating from animal or plant cells) or synthetic phospholipids of the cephalin or lecithin type such as e.g. phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerine, lysolecithin, cardiolipin, dioctanylephosphatidylcholine, dipalmitoylphosphatidylcholine and their mixtures.

Suitable non-ionic surfactants include polyethoxylated and polypropoxylated derivatives of alkylphenols, fatty alcohols, fatty acids, aliphatic amines or amides containing at least 12 carbon atoms in the molecule, alkylarenesulphonates and dialkylsulphosuccinates, such as

polyglycol ether derivatives of aliphatic and cycloaliphatic alcohols, saturated and unsaturated fatty acids and alkylphenols, said derivatives preferably containing 3 to 10 glycol ether groups and 8 to 20 carbon atoms in the (aliphatic) hydrocarbon moiety and 6 to 18 carbon atoms in the alkyl moiety of the alkylphenol. Further suitable non-ionic surfactants are water-soluble adducts of polyethylene oxide with polypropylene glycol, ethylenediaminopolypropylene glycol containing 1 to 10 carbon atoms in the alkyl chain, which adducts contain 20 to 250 ethyleneglycol ether groups and/or 10 to 100 propyleneglycol ether groups. Such compounds usually contain from 1 to 5 ethyleneglycol units per propyleneglycol unit. Representative examples of non-ionic surfactants are nonylphenol-polyethoxyethanol, castor oil polyglycolic ethers, polypropylene/ polyethylene oxide adducts, tributylphenoxypolyethoxyethanol, polyethyleneglycol and octylphenoxypolyethoxyethanol. Fatty acid esters of polyethylene sorbitan (such as polyoxyethylene sorbitan trioleate), glycerol, sorbitan, sucrose and pentaerythritol are also suitable non-ionic surfactants.

Suitable cationic surfactants include quaternary ammonium salts, preferably halides, having 4 hydrocarbon radicals optionally substituted with halo, phenyl, substituted phenyl or hydroxy; for instance quaternary ammonium salts containing as N-substituent at least one C<sub>8</sub>-C<sub>22</sub> alkyl radical (e.g. cetyl, lauryl, palmityl, myristyl, oleyl and the like) and, as further substituents, unsubstituted or halogenated lower alkyl, benzyl and/or hydroxy-lower alkyl radicals.

A more detailed description of surface-active agents suitable for this purpose may be found for instance in "McCutcheon's Detergents and Emulsifiers Annual" (MC Publishing Corp., Ridgewood, New Jersey, 1981), "Tensid-Taschenbuch", 2<sup>nd</sup> ed. (Hanser Verlag, Vienna, 1981) and "Encyclopaedia of Surfactants (Chemical Publishing Co., New York, 1981).

Structure-forming, thickening or gel-forming agents may be included into the pharmaceutical compositions and combined preparations of the invention. Suitable such agents are in particular highly dispersed silicic acid, such as the product commercially available under the trade name Aerosil; bentonites;

tetraalkyl ammonium salts of montmorillonites (e.g., products commercially available under the trade name Bentone), wherein each of the alkyl groups may contain from 1 to 20 carbon atoms; cetostearyl alcohol and modified castor oil products (e.g. the product commercially available under the trade name Antisettle).

Gelling agents which may be included into the pharmaceutical compositions and combined preparations of the present invention include, but are not limited to, cellulose derivatives such as carboxymethylcellulose, cellulose acetate and the like; natural gums such as arabic gum, xanthum gum, tragacanth gum, guar gum and the like; gelatin; silicon dioxide; synthetic polymers such as carbomers, and mixtures thereof. Gelatin and modified celluloses represent a preferred class of gelling agents.

Other optional excipients which may be included in the pharmaceutical compositions and combined preparations of the present invention include additives such as magnesium oxide; azo dyes; organic and inorganic pigments such as titanium dioxide; UV-absorbers; stabilisers; odor masking agents; viscosity enhancers; antioxidants such as, for example, ascorbyl palmitate, sodium bisulfite, sodium metabisulfite and the like, and mixtures thereof; preservatives such as, for example, potassium sorbate, sodium benzoate, sorbic acid, propyl gallate, benzylalcohol, methyl paraben, propyl paraben and the like; sequestering agents such as ethylene-diamine tetraacetic acid; flavoring agents such as natural vanillin; buffers such as citric acid and acetic acid; extenders or bulking agents such as silicates, diatomaceous earth, magnesium oxide or aluminum oxide; densification agents such as magnesium salts; and mixtures thereof.

Additional ingredients may be included in order to control the duration of action of the biologically-active ingredient in the compositions and combined preparations of the invention. Control release compositions may thus be achieved by selecting appropriate polymer carriers such as for example polyesters, polyamino-acids, polyvinyl-pyrrolidone, ethylene-vinyl acetate copolymers, methylcellulose, carboxymethylcellulose, protamine sulfate and the like. The rate of drug release and duration of action may also be controlled by incorporating the active ingredient into particles, e.g. microcapsules, of a

polymeric substance such as hydrogels, polylactic acid, hydroxymethyl-cellulose, polymethyl methacrylate and the other above-described polymers. Such methods include colloid drug delivery systems like liposomes, microspheres, microemulsions, nanoparticles, nanocapsules and so on.

- 5 Depending on the route of administration, the pharmaceutical composition or combined preparation of the invention may also require protective coatings.

Pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation thereof. Typical carriers for this purpose therefore include  
10 biocompatible aqueous buffers, ethanol, glycerol, propylene glycol, polyethylene glycol, complexing agents such as cyclodextrins and the like, and mixtures thereof.

Since, in the case of combined preparations including the pteridine derivative of this invention and an immunosuppressant or immunomodulator  
15 or antihistamine or antineoplastic drug or antiviral agent, both ingredients do not necessarily bring out their synergistic therapeutic effect directly at the same time in the patient to be treated, the said combined preparation may be in the form of a medical kit or package containing the two ingredients in separate but adjacent form. In the latter context, each ingredient may  
20 therefore be formulated in a way suitable for an administration route different from that of the other ingredient, e.g. one of them may be in the form of an oral or parenteral formulation whereas the other is in the form of an ampoule for intravenous injection or an aerosol.

The present invention further relates to a method for preventing or treating  
25 a disease selected from the group consisting of CNS disorders, cell proliferative disorders, allergic conditions, viral infections, immune and auto-immune disorders, transplant rejections and TNF- $\alpha$ -related disorders in a patient, preferably a mammal, more preferably a human being. The method of this invention consists of administering to the patient in need thereof an  
30 effective amount of a pteridine derivative having the general formula (I), optionally together with an effective amount of another immunosuppressant or immunomodulator or antineoplastic drug or antiviral agent, or a pharmaceutical composition comprising the same, such as disclosed above in

extensive details. The effective amount is usually in the range of 0.01 mg to 20 mg, preferably 0.1 mg to 5 mg, per day per kg bodyweight for humans. Depending upon the pathologic condition to be treated and the patient's condition, the said effective amount may be divided into several sub-units per day or may be administered at more than one day intervals. The patient to be treated may be any warm-blooded animal, preferably a human being, suffering from said pathologic condition.

The following examples are intended to illustrate several embodiments of the present invention, including the preparation of the pteridine derivatives and their pyrimidine intermediates, without limiting its scope in any way.

#### Example 1 – preparation of 2,6-diamino-5-nitroso-4-hydroxypyrimidine

The following illustrates the method step (a) shown in figure 1. To a solution of 2,6-diamino-4-hydroxypyrimidine (12.9 g, 102 mmoles) in 200 ml of a 10% acetic acid solution in water at 80 °C was added dropwise a solution of NaNO<sub>2</sub> (7.05 g, 102 mmoles) in 20 ml water. A pink precipitate was formed, which was further stirred for 1 hour at 80 °C. The reaction mixture was cooled down in the refrigerator overnight. The precipitate was filtered off and dried over P<sub>2</sub>O<sub>5</sub>, yielding the title compound as a pink powder (15.43 g, 97%). Its spectral data are in accordance with literature data (Traube in *Ber.* (1900) 33:1371 and Landauer et al. in *J. Chem. Soc.* (1953) 3721-3722).

#### Example 2 - synthesis of 2,5,6-triamino-4-hydroxypyrimidine

The following illustrates the method step (b) shown in figure 1. A suspension of the compound of example 1 (15 g, 96.7 mmoles) in an ammonium sulfide solution (20 % in water, 200 ml) was stirred overnight at 50 °C. The reaction mixture was cooled down in the refrigerator and the formed precipitate was filtered off, yielding the title compound as a yellow powder (11.33 g, 83 %). The spectral data are identical with literature data (same as for example 1).

Example 3 - synthesis of 3,4-dimethoxyphenylglyoxalmonoxime

In a mixture of dioxane (250 ml) and water (10 ml),  $\text{SeO}_2$  (0.33 mole) was heated to 50 °C. After solution of  $\text{SeO}_2$ , 3,4-dimethoxyacetophenone was added and the mixture heated under reflux for 16 hours. The hot solution was  
 5 filtered to remove selenium. The filtrate was evaporated, the oily residue dissolved in  $\text{CHCl}_3$  (300 ml), then washed with saturated  $\text{NaHCO}_3$  solution (100 ml) and water. The organic phase was dried over  $\text{Na}_2\text{S}_2\text{O}_4$ , filtered and evaporated. The yellow oil was distilled in vacuum, the resulting 3,4-dimethoxyphenylglyoxal was dissolved in MeOH (50 ml) and water (200 ml),  
 10 then acetoxime (0.25 mol) was added and the pH adjusted to 4 by 2 N HCl. The solution was heated to 50 °C for 2 hours, then cooled to 0 °C and the resulting crystals collected. After washing with cold water and drying in a vacuum desiccator, 3,4-dimethoxyphenylglyoxalmonooxime was obtained with a yield of 71 %. Recrystallization can be achieved from  $\text{CHCl}_3$  or acetone. The  
 15 compound was further characterized by nuclear magnetic resonance spectra as follows:  $^1\text{H}$ -NMR (200 MHz,  $\text{DMSO}-d_6$ ):  $\delta$  3.80 (3 H, s), 3.84 (3 H, s), 7.06 (1 H, d), 7.51 (1 H, s), 7.75 (1 H, d), 8.10 (1 H, s) and 12.51 (1 H, s) ppm.

Example 4 - synthesis of 2-amino-6-(3,4-dimethoxyphenyl) pterine

20 The following illustrates the method step (c) shown in figure 1. To a boiling solution of the compound of example 2 (2.4 g, 17 mmole) in methanol (100 ml, with 0.9 N HCl) was added dropwise a solution of the compound of example 3 (3.8 g, 18.2 mmole) in methanol (100 ml). The reaction mixture was heated under reflux for 4 hours. A precipitate was formed, which was filtered off,  
 25 washed with water, ethanol and diethyl ether. The precipitate was dried over  $\text{P}_2\text{O}_5$  under vacuum, yielding the title compound as a yellow powder (4.33 g, 85 %). This compound was further characterized by nuclear magnetic resonance spectra as follows:

- $^1\text{H}$ -NMR (500 MHz, TFA):  $\delta$  4.11 (3 H, s), 4.07 (3 H, s), 7.21 (1 H, d),  
 30 7.78 (1 H, dd), 7.81 (1 H, d) and 9.32 (1 H, s) ppm.
- $^{13}\text{C}$ -NMR (125 MHz, TFA):  $\delta$  at 56.39, 56.76, 111.94, 113.21, 123.22, 127.41, 127.91, 145.92, 149.39, 150.46, 152.47, 153.15, 155.13 and 161.59 ppm.

Example 5 - synthesis of 2-acetylamino-6-(3,4-dimethoxyphenyl) pterine

A suspension of the compound of example 4 (10.46 g, 35 mmole) in acetic anhydride (600 ml) and acetic acid (200 ml) was refluxed for 1 hour until a clear solution was formed. By cooling down the reaction mixture in a refrigerator, a precipitate was formed which was filtered off, washed with ethyl acetate and diethyl ether. The precipitate was dried over P<sub>2</sub>O<sub>5</sub> under vacuum, yielding the title compound as a yellow powder (9.19 g, 77 %). This compound was further characterized as follows:

- 10     - mass spectrum (MS): m/z (%): 300 ([M+H]<sup>+</sup>, 100)
- <sup>1</sup>H-NMR (200 MHz, DMSO-*d*<sub>6</sub>): δ 2.22 (3 H, s), 3.84 (3 H, s), 3.87 (3 H, s), 7.14 (1 H, d), 7.75 (2 H, m) and 9.51 (1 H, s) ppm.

Example 6 - synthesis of 2-acetylamino-4-(1,2,4-triazolyl)-6-[3,4-(dimethoxy-phenyl)] pteridine

The following illustrates the method step (f) shown in figure 1. To a solution of phosphorus oxychloride (1.68 ml, 18 mmole) and 1,2,4-triazole (4.96 g, 72 mmole) in dry pyridine (110 ml) was added the compound of example 5 (2.45 g, 7.2 mmole). The suspension was stirred at room temperature for 4 hours. The precipitate was filtered off, washed with pyridine, toluene and diethyl ether. The resulting solid was dried over P<sub>2</sub>O<sub>5</sub> under vacuum, affording the title compound as a yellow powder (2 g, yield: 80 %) characterized by mass spectrum data as follows: m/z (%): 392 ([M+H]<sup>+</sup>, 100).

Example 7 - synthesis of 2-acetylamino-4-(piperazin-1-yl)-6-(3,4-dimethoxy-phenyl) pteridine

The following illustrates the method step (g) shown in figure 1. To a suspension of the compound of example 6 (3.92 g, 10 mmole) in dioxane (200 ml) was added piperazine (1.29 g, 15 mmole). The suspension was stirred for 16 hours at room temperature. The precipitate was filtered off and washed with dioxane, ethanol and diethyl ether. The solid was dried over P<sub>2</sub>O<sub>5</sub> under vacuum, yielding the title compound as a yellow powder (3.48 g, 85 %).



Examples 8 to 21 - synthesis of 2-amino-4-(N-acyl-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridines

- The following illustrates the method step (h) shown in figure 1. To a suspension of the compound of example 7 (409 mg, 1 mmole) in dry pyridine (40 ml) was added under nitrogen a suitable carboxylic acid chloride (1.2 mmole). The suspension was stirred at room temperature for 24 hours. The solvent was concentrated *in vacuo* and the crude residue was dissolved in a mixture of CH<sub>3</sub>OH/20 % K<sub>2</sub>CO<sub>3</sub> in water (1:1). The solution was stirred at room temperature for 16 hours. Evaporation of the solvents *in vacuo*, followed by purification of the residue by preparative TLC (silica, using a CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> mixture (5:95) as the eluent) afforded the desired compound as a yellow powder. This procedure provided, with a yield ranging from 33 % to 75 % depending upon the carboxylic acid chloride used, the following pure final pteridine derivatives which were characterized by their mass spectrum MS and optionally by their <sup>1</sup>H-NMR (500 MHz, DMSO-*d*<sub>6</sub>) spectrum:
- 2-amino-4-(N-acetyl)piperazin-1-yl)-6-(3,4-dimethoxyphenyl)pteridine (example 8): MS 410 ([M+H]<sup>+</sup>;
  - 2-amino-4-[(N-propionyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 9): MS 424 ([M+H]<sup>+</sup>;
  - 2-amino-4-[(N-hexanoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 10): MS 466 ([M+H]<sup>+</sup>;
  - 2-amino-4-(N-benzoylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 11): MS 472 ([M+H]<sup>+</sup>; <sup>1</sup>H-NMR: δ at 3.65 (br s, 2 H), 3.80-3.90 (m, 8 H), 4.37 (br s, 4 H), 6.76 (br s, 2 H, NH<sub>2</sub>), 7.07 (d, 1 H), 7.48 (m, 5 H), 7.59 (br d, 1 H), 7.66 (dd, 1 H) and 9.31 (s, 1 H) ppm;
  - 2-amino-4-[N-(4-chlorobenzoyl)]piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 12): MS 506 ([M+H]<sup>+</sup>;
  - 2-amino-4-[(N-2-thiophenecarbonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 13): MS 478 ([M+H]<sup>+</sup>;
  - 2-amino-4-[(N-diethylcarbamoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 14): MS 467 ([M+H]<sup>+</sup>;
  - 2-amino-4-[(N-hydrocinnamoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 15): MS 500 ([M+H]<sup>+</sup>;

- 2-amino-4-[*N*-(4-cyanobenzoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 16): MS 497 ( $[M+H]^+$ ;
- 2-amino-4-[(*N*-phenoxyacetyl)-piperazin-1-yl]-6-(3,4-dimethoxy-phenyl) pteridine (example 17): MS 502 ( $[M+H]^+$ ;  $^1\text{H-NMR } \delta$  at 3.75 (br d, 4 H),  
5 3.83 (s, 3 H), 3.86 (s, 3 H), 4.32 (br s, 2 H), 4.41 (br s, 2 H), 4.90 (s, 2H), 6.77 (br s, 2 H), 6.94-6.97 (m, 3 H), 7.09 (d, 1 H), 7.28-7.31 (m, 2 H), 7.62 (d, 1 H), 7.67 (dd, 1 H) and 9.32 (s, 1 H) ppm ;
- 2-amino-4-[(*N*-4-butylbenzoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 18): MS 528 ( $[M+H]^+$ ;
- 2-amino-4-[(*N*-isonicotinoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 19): MS 473 ( $[M+H]^+$ ;  $^1\text{H-NMR } \delta$  at 3.58 (br s, 2 H),  
10 3.82 (s, 6 H), 3.87 (br s, 2 H), 4.33 (br s, 2 H), 4.40 (br s, 2 H), 6.77 (br s, 2 H), 7.07 (d, 1 H), 7.47 (dd, 2 H), 7.59 (br d), 7.66 (dd, 1 H), 8.71 (dd, 2 H) and 9.32 (s, 1 H) ppm;
- 2-amino-4-[(*N*-diisopropylcarbamoyl)-piperazin-1-yl]-6-(3,4-dimethoxy-phenyl) pteridine (example 20): MS 495 ( $[M+H]^+$ ; and
- 2-amino-4-[*N*-(4-pentoxybenzoyl)-piperazin-1-yl]-6-(3,4-dimethoxy-phenyl) pteridine (example 21): MS 558 ( $[M+H]^+$ .

20 Example 22 - synthesis of 2,6-diamino-4-chloro-5-*p*-chlorophenylazopyrimidine

The following illustrates the method step (a) shown in figure 2. A solution of *p*-chloroaniline (25.5 g, 0.2 mole) in 6 N HCl (100 ml) was cooled to 0 °C and then NaNO<sub>2</sub> (13.8 g, 0.2 mole) in water (40 ml) was added dropwise  
25 with stirring. After the addition was completed, the solution was stirred for another 30 minutes. Urea (5 g) was added to destroy the excess of HNO<sub>2</sub>. The diazonium salt solution was then poured into a solution of 2,6-diamino-4-chloropyrimidine (26 g, 0.18 mole) in water (500 ml) and stirred for 30 minutes. Then potassium acetate (70 g) was added and the mixture was  
30 stirred for 16 hours at room temperature. The resulting precipitate was collected by suction, washed with water and dried in a vacuum dessicator over P<sub>2</sub>O<sub>5</sub> to give 44 g (81 %) of a yellow solid. Recrystallization can be

achieved from DMF/H<sub>2</sub>O. The spectral data are in analogy with those described in literature (Fröhlich et al. in *J. Med. Chem.* (1999) 42:4108-4121).

Example 23 - synthesis of 2,6-diamino-4-(piperazin-1-yl)-5-*p*-chlorophenylazo-pyrimidine

5 The following illustrates the method step (b) shown in figure 2. A solution of the compound of example 22 (5 g, 16.6 mole) in DMF (50 ml) and piperazine (10 g) was heated in an oilbath to 70 °C for 5 hours. Water (50 ml) was added and the reaction mixture was cooled down. The yellow precipitate  
10 was filtered off, washed with water and dried. Recrystallization can be achieved from ethanol.

Example 24 - synthesis of 2,5,6-triamino-4-(piperazin-1-yl) pyrimidine

The following illustrates the method step (c) shown in figure 2. To a  
15 suspension of the compound of example 23 (2.03 g, 6.1 mmole) in ethanol (98 ml) and water (98 ml) was added zinc (4.04 g) and acetic acid (2.02 ml). The suspension was refluxed until a clear solution was obtained, i.e. for 1 hour. Zinc was filtered off and the filtrate was evaporated, followed by co-evaporation with toluene. A brown precipitate was obtained, which was  
20 resuspended in diethyl ether and stirred overnight at room temperature in order to remove *p*-chloroaniline. The precipitate was filtered off and dried in a vacuum dessicator over P<sub>2</sub>O<sub>5</sub>.

Example 25 - synthesis of 2-amino-4-(piperazin-1-yl)-6-(3,4-dimethoxyphenyl)pteridine

25 The following illustrates the method step (d) shown in figure 2. To a solution of the compound of example 24 (2.65 mmole, 554 mg) in methanol (30 ml) was added 3,4-dimethoxyphenylglyoxaloxime (2.65 mmole, 554 mg). The pH of the reaction mixture was adjusted to 3 by adding a few drops of  
30 concentrated hydrochloric acid. The mixture was refluxed for 3 hours. A yellow precipitate was formed. The reaction mixture was cooled down and neutralized by the addition of concentrated NH<sub>3</sub> till pH 9. The precipitate was filtered off and used for further reaction without any purification.

Examples 26 to 32 - synthesis of 2-amino-4-(*N*-acylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridines and 2-amino-4-(*N*-sulfonylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridines

5        The following illustrates the method step (g) shown in figure 2. To a suspension of the crude compound of example 25 (506 mg, 1.38 mmole) in pyridine (30 ml) was added a suitable carboxylic acid chloride or sulfonyl chloride (2.07 mmole). The suspension was stirred at room temperature overnight. After pyridine evaporation, the residue was purified by flash  
10       chromatography on silica gel, the mobile phase being CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> mixtures (in a ratio gradually ranging from 2:98 to 5:95), optionally followed by preparative TLC on silica gel (the mobile phase being a 7:93 CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> mixture), thus affording the desired compound as a yellow powder. This procedure provided, with a yield ranging from 30 % to 50 % depending upon  
15       the carboxylic acid or sulfonyl chloride used, the following pure final pteridine derivatives which were characterized by their mass spectrum MS and optionally by their <sup>1</sup>H-NMR (200 MHz, DMSO-*d*<sub>6</sub>) spectrum:

- 2-amino-4-[*N*-(3-methoxybenzoyl)piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 26): MS 502 ([M+H]<sup>+</sup>; <sup>1</sup>H-NMR: 3.33 (br s, 4  
20       H), 3.80 (s, 9 H), 4.37 (br s, 4 H), 6.78 (br s, 2 H), 6.99-7.70 (m, 7 H) and 9.33 (s, 1 H) ppm.
- 2-amino-4-[*N*-(2-furoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 27): MS 462 ([M+H]<sup>+</sup>;
- 2-amino-4-[*N*-(benzyloxyacetyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 28): MS 516 ([M+H]<sup>+</sup>;  
25       pteridine (example 28): MS 516 ([M+H]<sup>+</sup>;
- 2-amino-4-[*N*-(*p*-chlorophenoxyacetyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 29): MS 536 ([M+H]<sup>+</sup>;
- 2-amino-4-[*N*-cyclohexylcarbonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 30): MS 478 ([M+H]<sup>+</sup>;
- 2-amino-4-[*N*-phenylsulfonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 31): MS 508 ([M+H]<sup>+</sup>; <sup>1</sup>H-NMR: 3.14 (br s, 4 H), 3.84  
30       (s, 3 H), 3.87 (s, 3 H), 4.40 (br s, 4 H), 6.88 (br s, 2 H), 7.09 (d, 1 H) and 7.56-7.78 (m, 7 H) ppm ; and

2-amino-4-[(*N*-*p*-fluorobenzoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 32): MS 490 ( $[M+H]^+$ ).

Example 33 - synthesis of 2,5,6-triamino-4-hydroxypyrimidine dihydrochloride

5        The following illustrates the method step (a) shown in figure 3. To a stirred suspension of 2,4,5-triamino-6-hydroxy-pyrimidine (40.93 g, 290 mmole) in methanol (500 ml) was added dropwise concentrated hydrochloric acid 37% (60.5 ml, 725 mmole). The suspension was stirred 30 minutes at room temperature, filtered and the precipitate was washed with methanol,  
10       diethyl ether and dried over KOH under vacuum, affording the title compound as a white powder (53.82 g, yield 88 %). Spectral data are identical with literature data (W. Pfeleiderer, *Chem. Ber.* (1957) 90:2272).

Example 34 - synthesis of 2-amino-6-(3,4-dimethoxyphenyl)pterine

15       The following illustrates the method step (b) shown in figure 3. The compound of example 33 (21.5 g, 102 mmole) and 3,4-dimethoxyphenyl-glyoxalmonooxime (25.61 g, 122.4 mmole) were suspended in methanol (400 ml) and the orange suspension was heated under reflux for 2.5 hours. The yellow suspension was cooled with an ice bath and the precipitate was filtered  
20       and successively washed with methanol, ethyl acetate, diethyl ether, and dried at 110°C for 3 hours to afford a shiny yellow powder (21.56 g, yield 71 %) which was used without further purification.

Example 35 - preparation of 2-amino-4-(piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine

25       The following illustrates the method step (e) shown in figure 3. Piperazine (12.06 g, 140 mmole) was added to a stirred suspension of the compound of example 34 (5.99 g, 20 mmole) in pyridine (64 ml) and 1,1,1,3,3,3-hexamethyldisilazane (64 ml, 300 mmole) in the presence of a  
30       catalytic amount of ammonium sulfate and *p*-toluenesulfonic acid. The mixture was heated under reflux for 15 hours and the brown solution was cooled with an ice bath. Methanol was added and the mixture was evaporated to dryness. The brown residue was co-evaporated 2 times with xylene, and adsorbed on

silica gel. The compound was purified on silica gel column chromatography, using a 9/1 CH<sub>2</sub>Cl<sub>2</sub>/MeOH mixture containing 1 % concentrated aqueous ammonia as eluent, thus affording the desired compound (3.12 g, yield 42 %) which was characterized by its mass spectrum MS and <sup>1</sup>H-NMR (200 MHz,

5 DMSO-*d*<sub>6</sub>) spectrum as follows:

- MS: *m/z* (%): 368 ([M+H]<sup>+</sup>, 100); and
- <sup>1</sup>H-NMR (200 MHz, DMSO-*d*<sub>6</sub>): δ 2.92 (4 H, br s), 3.83 (3 H, s), 3.86 (3 H, s), 4.27 (4 H, br s), 6.70 (2 H, br s), 7.08 (1 H, d), 7.62-7.69 (2 H, m) and 9.30 (1 H, s) ppm.

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Examples 36 to 51 - synthesis of 2-amino-4-(*N*-acylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridines, 2-amino-4-(*N*-sulfonylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridines and 2-amino-4-(*N*-thioacylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridines

15

The following illustrates the method step (f) shown in figure 3. To a suspension of the compound of example 35 (184 mg, 0.5 mmole) in CH<sub>2</sub>Cl<sub>2</sub> (10 ml) was added triethylamine (84 µl, 0.6 mmole) and a suitable carboxylic acid, thiocarboxylic or sulfonyl chloride (0.55 mmole). The suspension was stirred at room temperature for 2 to 24 hours. The solution was diluted CH<sub>2</sub>Cl<sub>2</sub> (20 ml) and extracted with a 5% aqueous solution of sodium hydrogen carbonate (30 ml). The organic layer was concentrated *in vacuo* and the crude residue was subjected to silica gel column chromatography, the mobile phase being CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> mixtures (in a ratio gradually ranging from 1:99 to 5:95), thus affording the desired compound as a yellow powder. This procedure provided, with a yield ranging from 35 % to 85 % depending upon the carboxylic acid, thiocarboxylic or sulfonyl chloride used, the following pure final pteridine derivatives which were characterized by their mass spectrum MS:

20

25

- 2-amino-4-[(*N*-2-thiophenacetyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 36): MS 492 ([M+H]<sup>+</sup>;
- 2-amino-4-[(*N*-cinnamoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 37): MS 498 ([M+H]<sup>+</sup>;

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- 2-amino-4-[(*N*-1-pyrrolidinylcarbonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 38): MS 951 ([2M+Na]<sup>+</sup>, 15); 929 ([2M+H]<sup>+</sup>, 15); 465 ([M+H]<sup>+</sup>, 100)
- 2-amino-4-[(*N*-diphenylcarbonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 39): MS 563 ([M+H]<sup>+</sup>;
- 2-amino-4-[*N*-(2,6-dichloro-5-fluoro-nicotinoyl)]-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 40): MS 559 ([M+H]<sup>+</sup>;
- 2-amino-4-[(*N*-methoxyacetyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 41): MS 901 ([2M+Na]<sup>+</sup>, 20); 879 ([2M+H]<sup>+</sup>, 10) and 440 ([M+H]<sup>+</sup>, 100);
- 2-amino-4-[*N*-(2-methoxybenzoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 42): MS 502 ([M+H]<sup>+</sup>;
- 2-amino-4-[(*N*-benzylsulfonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 43): MS 522 ([M+H]<sup>+</sup>;
- 2-amino-4-[*N*-(3,4-dichlorobenzoyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 44): MS 540 ([M+H]<sup>+</sup>;
- 2-amino-4-[*N*-(4-chlorophenylacetyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 45): MS 520 ([M+H]<sup>+</sup>;
- 2-amino-4-[(*N*-(1-naphtoyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 46): MS 522 ([M+H]<sup>+</sup>;
- 2-amino-4-[*N*-(3-furoylcarbonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 47): MS 490 ([M+H]<sup>+</sup>;
- 2-amino-4-[(*N*-benzyloxycarbonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 48): MS 502 ([M+H]<sup>+</sup>;
- 2-amino-4-[(*N*-dimethylthiocarbonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 49): MS 455 ([M+H]<sup>+</sup>;
- 2-amino-4-[(*N*-phenoxyacetyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 50): MS 487 ([M+H]<sup>+</sup>; and
- 2-amino-4-[(*N*-phenoxythiocarbonyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 51): MS 504 ([M+H]<sup>+</sup>.

Example 52 - synthesis of 2,6-diamino-4-(*N*-acetyl)piperazin-1-yl)-pyrimidine

The following illustrates the method step (a) shown in figure 4. N-acetylpiperazine (12.82 g, 100 mmole) was added to a stirred suspension of 4-chloro-2,6-diaminopyrimidine (7.23 g, 50 mmole, m.p. 199°C, commercially available for instance from Merck or from Qiaoji Group Co. Ltd., Hong-Kong) in water (100 ml), and the mixture was refluxed for 21 hours. The orange solution was cooled and made alkaline with NaOH 10M (5 ml) to lead to a white precipitation. The solution was filtered; the solid was washed with cold water and dried over P<sub>2</sub>O<sub>5</sub> in a vacuum dessicator to afford the desired compound as a white powder (9.77 g, yield 82%) which was characterized by the following mass spectrum MS *m/z* (%): 237 ([M+H]<sup>+</sup>, 100); 195 ([M-Ac+H]<sup>+</sup>, 25).

Example 53 - synthesis of 2,6-diamino-5-nitroso-4-(N-acetylpiperazin-1-yl)-pyrimidine

The following illustrates the method step (b) shown in figure 4. Acetic acid (4 ml) was added dropwise to a stirred suspension of the compound of example 52 (9.45 g, 40 mmole) and sodium nitrite (3.04 g, 44 mmole) in water (200 ml) at room temperature. The purple mixture was stirred for 1 hour and cooled down to 5°C for 14 hours. The precipitate was filtered, washed with water, diethyl ether and dried over P<sub>2</sub>O<sub>5</sub> in a vacuum dessicator to afford the titled compound as a purple powder (10.53 g, yield 99%) which was characterized by the following mass spectrum MS *m/z* (%): 288 ([M+Na]<sup>+</sup>, 60); 266 ([M+H]<sup>+</sup>, 100).

Example 54 - synthesis of 2,5,6-triamino-4-(N-acetylpiperazin-1-yl)-pyrimidine

The following illustrates the method step (c) shown in figure 4. To a suspension of the compound obtained in example 53 (1 g, 3.77 mmole) in water (25 ml) was added sodium dithionite (1.97 g, 11.3 mmole). The suspension was heated to 50 °C till a clear solution was obtained. Water was evaporated *in vacuo* and the residue was co-evaporated with toluene twice. The crude material was used for further reaction without any purification.

Example 55 - synthesis of 2-amino-4-(N-acetylpiperazin-1-yl)-6-phenylpteridine



The following illustrates the method step (d) shown in figure 4. The crude product obtained in example 54 and isonitrosoacetophenone (653 mg, 4.0 mmole) were suspended in a 1.25 M HCl solution in MeOH (20 ml) and the mixture was refluxed for 3 hours. The reaction mixture was cooled down to room temperature and neutralized with NH<sub>3</sub> 25 % aqueous solution to pH 9. The mixture was evaporated to dryness and the residue was partitioned between CHCl<sub>3</sub> and H<sub>2</sub>O. The organic layer was separated, evaporated to dryness and purified by silica gel chromatography, the mobile phase consisting of CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> mixtures (in a ratio gradually ranging from 2:98 to 4:96), thus affording the desired compound as a yellow powder (978 mg, yield 70 %) was characterized by its mass spectrum MS as follows: MS *m/z* (%): 721 ([2M+Na]<sup>+</sup>, 60); 372 ([M+Na]<sup>+</sup>, 10) and 350 ([M+H]<sup>+</sup>, 100).

Example 56 - synthesis of 2-amino-4-(N-acetylpiperazin-1-yl)-6-(4-tolyl) pteridine

The method of example 55 was repeated, except for using 4-methylphenylglyoxalmonoxime (4.0 mmole) instead of isonitrosoacetophenone. This afforded the title compound as a yellow powder (900 mg, yield 62 %), which was characterized by its mass spectrum MS as follows: MS *m/z* (%): 362 ([M+H]<sup>+</sup>, 100).

Example 57 - synthesis of 2-amino-4-(N-acetylpiperazin-1-yl)-6-(4-fluorophenyl) pteridine

The following illustrates the method step (d) shown in figure 4. The crude product obtained in example 54 was dissolved in a 1.25 M HCl solution in MeOH (20 ml) and 4-fluorophenylglyoxalmonoxime (504 mg, 3.0 mmole) was added portionwise. The mixture was refluxed for 3 hours. The reaction mixture was cooled down to room temperature and neutralized with NH<sub>3</sub> 25 % aqueous solution to pH 9. The yellow precipitate was filtered off and washed with water. The precipitate was adsorbed on silica gel and purified by flash chromatography, the mobile phase consisting of CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> mixtures (in a ratio gradually ranging from 1:99 to 4:96), thus affording the title compound as

a yellow powder (734 mg, 53 % yield), which was characterized by its mass spectrum MS as follows: MS  $m/z$  (%): 368 ( $[M+H]^+$ , 100).

5 Example 58 - synthesis of 2-amino-4-(*N*-acetylpiperazin-1-yl)-6-(4-chloro-phenyl) pteridine

The method of example 57 was repeated, except for using 4-chlorophenylglyoxalmonoxime (554 mg, 3.0 mmole) instead of 4-fluorophenylglyoxalmonoxime. This afforded the title compound as a yellow powder (924 mg, 64 % yield), which was characterized by its mass spectrum MS as follows: MS  $m/z$  (%): 384 ( $[M+H]^+$ , 100).

Example 59 - synthesis of 2-amino-4-(*N*-acetylpiperazin-1-yl)-6-(4-acetamidophenyl) pteridine

The method of example 57 was repeated, except for using 4-acetylbenzamido-phenylglyoxalmonoxime (206 mg, 3.0 mmole) instead of 4-fluorophenylglyoxalmonoxime, and performing silica gel flash chromatography with a  $CH_3OH/CH_2Cl_2$  gradient from 2:98 to 10:90. This afforded the title compound as a yellow powder (871 mg, 57 % yield), which was characterized by its mass spectrum MS as follows: MS  $m/z$  (%): 407 ( $[M+H]^+$ , 100).

20 Examples 60 to 63 - synthesis of 2-amino-4-[*N*-( $\alpha$ -aminoacyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridines

The following illustrates the method step (a) shown in figure 5, wherein  $R_{11}$  is a radical having an amino group in  $\alpha$  position with respect to the carboxylic acid group. The compound of example 35 (0.367 g, 1 mmole) and a tert-butoxycarbonyl-protected amino-acid, such as L-phenylalanine (example 60), L-tyrosine (example 61), L-proline (example 62) or L-tryptophane (example 63), were suspended in dry dimethylformamide at room temperature under nitrogen and diisopropylethylamine (0.418 ml, 2.4 mmole), followed by *o*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (0.482 g, 1.5 mmole) were added. The mixture was stirred for 2 hours and diluted with dichloromethane (50 ml). The organic layer was washed with a saturated solution of sodium hydrogen carbonate (50 ml), dried over anhydrous sodium sulfate and evaporated to dryness. The crude residue was purified by silica

gel chromatography, the mobile phase consisting of CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> mixtures (in a ratio gradually ranging from 3:97 to 6:94), with 0.5% concentrated aqueous ammonia if needed. This procedure provided tert-butoxycarbonyl-protected 2-amino-4-[N-( $\alpha$ -aminoacyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine intermediates with yields ranging from 50 % to 80 % depending upon the tert-butoxycarbonyl-protected amino-acid used.

Then the said tert-butoxycarbonyl-protected intermediate (0.5 mmole) was suspended in a mixture of dioxane (10 ml) and HCl 6M (20 ml) and stirred at room temperature until complete mixture. The medium was neutralized with NaOH 10M and volatiles were removed. The residue was adsorbed on silica and purified by silica gel column chromatography, the mobile phase consisting of CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> mixtures (in a ratio gradually ranging from 4:96 to 6:94) containing 0.5% of concentrated aqueous ammonia.

This procedure provided, with a yield ranging from 50 % to 70 % depending upon the tert-butoxycarbonyl-protected amino-acid used, the following pure final pteridine derivatives as yellow powders which were characterized by their mass spectrum MS as follows:

- 2-(S)-amino-4-[N-(2-amino-3-phenylpropionyl)-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 60): MS 515 ([M+H]<sup>+</sup>;
- 2-(S)-amino-4-[N-[2-amino-3-(4-hydroxyphenyl)propionyl]-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 61): MS 531 ([M+H]<sup>+</sup>;
- 2-(S)-amino-4-[N-(pyrrolidin-2-yl)carbonyl-piperazin-1-yl]-6-(3,4-dimethoxy-phenyl) pteridine (example 62): MS 465 ([M+H]<sup>+</sup>; and
- 2-(S)-amino-4-[N-2-amino-3-(indol-2-yl)propionyl]-piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 63): MS 554 ([M+H]<sup>+</sup>.

#### Example 64 - synthesis of 2-amino-4-(N-phenylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine

To a suspension of the compound of example 6 (196 mg, 0.5 mmole) in dioxane (10 ml) was added N-phenylpiperazine (0.23 ml, 1.5 mmole). The suspension was stirred at room temperature overnight. The precipitate was filtered off and washed with dioxane and diethylether, yielding the crude 2-acetyl-amino-4-(N-phenylpiperazine)-6-(3,4-dimethoxyphenyl) pteridine).

Deprotection of the acetylamino group was achieved by dissolving this crude compound in methanol (5 ml) and a 20 %  $K_2CO_3$  solution in water (5 ml). The solution was stirred overnight. Solvents were evaporated *in vacuo* and the residue was purified by preparative TLC (silica, using a  $CH_3OH/CH_2Cl_2$  (5:95) mixture as an eluent), affording the title compound as a yellow powder (84 mg, yield 38 %) which was characterized by its mass spectrum MS as follows: MS  $m/z$  (%): 444 ( $[M+H]^+$ , 100).

10 Example 65 - synthesis of 2-amino-4-(N-benzylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine

Repeating the method of example 64, except for using *N*-benzylpiperazine (0.26 ml, 1.5 mmole) instead of *N*-phenylpiperazine, afforded the title compound as a yellow powder (75 mg, yield 33%) which was characterized by its mass spectrum MS as follows: MS  $m/z$  (%): 458 ( $[M+H]^+$ , 100).

15

Example 66 - synthesis of 2-amino-4-(N-trans-cinnamylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine

Repeating the method of example 64, except for using *N*-cinnamylpiperazine (0.306 ml, 1.5 mmole) instead of *N*-phenylpiperazine, afforded the title compound as a yellow powder (99 mg, yield 41%) which was characterized by its mass spectrum MS as follows: MS  $m/z$  (%): 484 ( $[M+H]^+$ , 100).

20

Examples 67 to 70 - synthesis of 2-substituted 4,6-diamino-5-nitroso-pyrimidines

25 To a suspension of 4,6-diamino-2-methylmercapto-5-nitroso-pyrimidine (1 g, 5.41 mmole), which may be prepared and characterised for instance as disclosed by Baddiley et al. in J. Chem. Soc. (1943) 383, in water (25 ml) was added a large excess (162 mmole) of an appropriate secondary amine. After heating the reaction mixture at 65 °C during 3 hours, a pink suspension was formed. The reaction mixture was then cooled down to + 4°C for 4 days. The pink precipitate was filtered off and washed with water, yielding the pure following compounds, each being characterised by its mass spectrum (MS), in yields ranging from 30 to 50 % :

30

- 2-phenylethylamino-4,6-diamino-5-nitroso-pyrimidine (example 67) was obtained from phenylethylamine ; MS: m/z (%): 259 ( $[M+H]^+$ , 100).
- 2-(2-thienylmethylamino)-4,6-diamino-5-nitroso-pyrimidine (example 68) was obtained from 2-thiophenemethylamine ; MS: m/z (%): 251 ( $[M+H]^+$ , 100).
- 2-pyrrolidino-4,6-diamino-5-nitroso-pyrimidine (example 69) was obtained from pyrrolidine ; MS: m/z (%): 209 ( $[M+H]^+$ , 100).
- 2-benzylamino-4,6-diamino-5-nitroso-pyrimidine (example 70) was obtained from benzylamine ; MS: m/z (%): 245 ( $[M+H]^+$ , 100).

Example 71 to 74 - synthesis of 2-substituted-4,5,6-triamino-pyrimidine sulfates

To a suspension of a 2-substituted-4,6-diamino-5-nitroso-pyrimidine obtained in one of examples 67 to 70 (1 mmole) in water (25 ml) was added portionwise sodium dithionite (3 mmole). The resulting suspension was refluxed until a yellow solution was formed. A sulfuric acid solution (2.5 ml of a 50 % solution in water) was then added. The reaction mixture was cooled down to + 4°C for 5 hours. The white precipitate formed was filtered off, yielding the pure following compounds in yields ranging from 60% to 75%.

- 2-phenylethylamino- 4,5,6-triamino-pyrimidine sulfate (example 71),
- 2-(2-thienylmethylamino)- 4,5,6-triamino-pyrimidine sulfate (example 72),
- 2-pyrrolidino- 4,5,6-triamino-pyrimidine sulfate (example 73), and
- 2-benzylamino- 4,5,6-triamino-pyrimidine sulfate (example 74).

Examples 75 to 78 - synthesis of 2-substituted-4,5,6-triamino-pyrimidine dihydrochlorides

To a suspension of a 2-substituted-4,5,6-triamino-pyrimidine sulfate obtained in one of examples 71 to 74 (1 mmole) in water (6 ml) at 80 °C was added dropwise a solution of barium chloride dihydrate (0.9 mmole) in water (2 ml). The resulting suspension was stirred for 30 minutes at 80 °C, then the reaction mixture was cooled down and barium sulfate was filtered off over Celite. The filtrate was evaporated in vacuo and co-evaporated with toluene

yielding each of the following compounds as a yellow powder in yields ranging from 90 % to 98 %:

- 2-phenylethylamino- 4,5,6-triamino-pyrimidine dihydrochloride (example 75),
- 5 - 2-(2-thienylmethylamino)- 4,5,6-triamino-pyrimidine dihydrochloride (example 76),
- 2-pyrrolidino- 4,5,6-triamino-pyrimidine dihydrochloride (example 77), and
- 2-benzylamino- 4,5,6-triamino-pyrimidine dihydrochloride (example 78).

10 Examples 79 to 82 - synthesis of 2-substituted-4-amino-6-(3,4-dimethoxy-phenyl) pteridines

The following procedure is in accordance with figure 6. To a solution of a 2-substituted-4,5,6-triamino-pyrimidine dihydrochloride obtained in one of examples 75 to 78 (1 mmole) in methanol (15 ml) was added the 3,4-dimethoxyphenylglyoxaloxime obtained according to example 3 (1 mmole).  
 15 The resulting solution was refluxed for 2 hours, thus forming a yellow suspension. The reaction mixture was cooled down and neutralised by addition of a 33% aqueous ammonia solution until pH 9 was reached. The yellow precipitate was filtered off and further purified by silica gel flash  
 20 chromatography (eluting with solvent mixture CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub>, gradient from 1:99 to 3:97), yielding as a yellow powder each of the pure following compounds, which was characterised by its mass spectrum (MS) and its ultraviolet spectrum (UV):

- 2-phenylethylamino-4-amino-6-(3,4-dimethoxyphenyl) pteridine (example 25 79) was obtained from the salt of example 75; MS: m/z (%): 403 ([M+H]<sup>+</sup>, 100), 827 ([2M+Na]<sup>+</sup>, 20); UV (MeOH, nm): 287, 315, 412.
- 2-(2-thienylmethylamino)- 4-amino-6-(3,4-dimethoxyphenyl) pteridine (example 80) was obtained from the salt of example 76; MS: m/z (%): 394 ([M+H]<sup>+</sup>, 100); UV (MeOH, nm): 287, 314, 410.
- 30 - 2-pyrrolidino-4-amino-6-(3,4-dimethoxyphenyl) pteridine (example 81) was obtained from the salt of example 77; MS: m/z (%): 353 ([M+H]<sup>+</sup>, 100), 727 ([2M+Na]<sup>+</sup>, 10); UV (MeOH, nm): 319, 423.

- 2-benzylamino-4-amino-6-(3,4-dimethoxyphenyl) pteridine (example 82) was obtained from the salt of example 78; MS: m/z (%): 389 ( $[M+H]^+$ , 100); UV (MeOH, nm): 287, 315, 411.

5 Example 83 - synthesis of 4,5,6-triamino-2-methylmercaptopyrimidine dihydrochloride

To a suspension of a 2-methylmercapto-4,5,6-triamino-pyrimidine sulfate (44.3 mmole), which may be prepared and characterised for instance as disclosed by Taylor et al. in J. Am. Chem. Soc. (1952) 74:1644-1647, in  
 10 water (135 ml) at 80 °C was added dropwise a solution of barium chloride dihydrate (39.8 mmole) in water (25 ml). The suspension was stirred for 30 minutes at 80 °C. The reaction mixture was cooled down and barium sulfate was filtered off over Celite. The filtrate was evaporated in vacuo and co-evaporated with toluene yielding the title compound as a yellow powder (10.2  
 15 g, 94 % yield).

Example 84 - synthesis of 4-amino-2-methylmercapto-6-(3,4-dimethoxyphenyl) pteridine

To a suspension of 4,5,6-triamino-2-methylmercaptopyrimidine dihydrochloride (7.42 mmole, 1.81 g) in methanol (20 ml) was added a  
 20 solution of 3,4-dimethoxyphenylglyoxaloxime (5.94 mmole, 1.24 g) in methanol. The resulting reaction mixture was refluxed for 3 hours. The reaction mixture was neutralised with concentrated aqueous ammonia until pH 9 was reached. The resulting precipitate was filtered off and further purified by  
 25 flash chromatography (silica, using an ethyl acetate / hexane mixture in a 4:6 ratio) yielding the pure title compound as a yellow powder which was characterised as follows: MS: m/z (%) 330 ( $[M+H]^+$ , 100), 681 ( $[2M+Na]^+$ , 30); UV (MeOH, nm): 292, 397.

30 Example 85 - synthesis of 4-amino-2-methylmercapto-6-phenyl-pteridine

A method similar to that of example 84 was used, starting from phenylglyoxal monoxime instead of 3,4-dimethoxyphenylglyoxalmonoxime.

The title compound was characterised as follows: MS:  $m/z$  (%): 270 ( $[M+H]^+$ , 100); UV (MeOH, nm): 286, 379.

Example 86 - synthesis of 4-amino-2-morpholino-6-(3,4-dimethoxyphenyl) pteridine

5 A solution of the compound of example 84 (100 mg, 0.304 mmole) in morpholine (12 ml) was refluxed overnight. The solvents were removed in vacuo and the residue was purified first by flash chromatography (silica, gradient from 2:98 to 3:97  $CH_3OH/CH_2Cl_2$ ) and then by preparative TLC  
10 (silica, EtOAc/hexane 1 :1) yielding the title compound as a yellow powder (70 mg, 63 % yield) characterised as follows: MS:  $m/z$  (%): 369 ( $[M+H]^+$ , 100), 759 ( $[2M+Na]^+$ , 20); UV (MeOH, nm): 297, 315, 418.

Example 87 - synthesis of 4-amino-2-piperidino-6-(3,4-dimethoxyphenyl) pteridine

15 A method similar to that of example 86 was used, starting from piperidine instead of morpholine. The title compound obtained as a yellow powder (58 mg, 52 %) was characterised as follows: MS:  $m/z$  (%): 367 ( $[M+H]^+$ , 100), 755 ( $[2M+Na]^+$ , 10); UV (MeOH, nm): 319, 425.

20 Example 88 - synthesis of 2,4-di-(thienyl-2-methylamino)-6-(3,4-dimethoxyphenyl) pteridine

A method similar to that of example 86 was used, starting from thiophene-methylamine instead of morpholine. The title compound obtained  
25 as a yellow powder (85 mg, 57 %) was characterised as follows: MS:  $m/z$  (%): 491 ( $[M+H]^+$ , 100); UV (MeOH, nm): 229, 296, 414.

Example 89 - synthesis of 2,5,6-triamino-4-morpholino-pyrimidine dihydrochloride

30 In a 10 L 4-neck flask equipped with a mechanical stirrer, a thermometer and a heating mantle was placed 2,6-diamino-4-chloropyrimidine (870.5 g; 6.0 mol) in water (3190 ml). To the stirred resulting white suspension was added morpholine (1113 g; 12.8 moles) in one portion. The temperature



raised from 14 to 28°C and the mixture was heated until at 75°C a clear solution was obtained and refluxed for 16 hours. After complete conversion of the starting material (controlled by HPLC analysis) the reaction mixture was cooled to room temperature and NaNO<sub>2</sub> (465 g, 6.74 mole) was added in three portions. No heat effect was observed but the product precipitated out of the mixture. During subsequent dropwise addition of acetic acid (600 g, 9.0 moles) the mixture became so thick that faster stirring and additional water (850 mL) were necessary to achieve a well mixable suspension. A temperature raise from 15 to 28°C was measured. After stirring for 2 hours, HPLC analysis showed complete conversion. The mixture was then allowed to cool down to room temperature and transferred to a 20L flask. To the acid (pH 4) purple slurry was added water (3500 mL) and then an aqueous NaOH solution (165 g in 825 mL) in order to neutralise to pH 7. The suspension was cooled down to 10-12°C in an ice-water bath and Na<sub>2</sub>S<sub>2</sub>O<sub>4</sub> (4.68 kg, 26.9 moles) was added in portions of about 500 g at 5 minutes intervals. A temperature raise to 30°C was observed. The mixture was left stirring overnight at room temperature before being analysed (HPLC: 99.3% conversion). The suspension was filtered over filter cloth and the wet cake was washed with 2.77 L water leaving 1.5 kg of wet material which was slurried in isopropanol (10.2 L) and to the suspension was added dropwise 36% HCl/H<sub>2</sub>O (1.16L) during which the temperature raised from 15 to 30°C. Subsequent heating of the slurry to 40°C afforded an almost clear solution and crystallisation started suddenly showing an exotherm to 50°C. The suspension was cooled down to room temperature before the suspension was filtered over filter cloth. The wet cake was again slurried in isopropanol (5.0 L), again filtered over filter cloth and dried at 40°C in vacuo (200 mbar) for several days. According to this procedure was obtained 1.2 kg (yield 54.1%) of 2,5,6-triamino-4-morpholino-pyrimidine dihydrochloride with a purity above 99.9% and characterised as follows: UV (MeOH, nm): 225, 254.

#### Example 90 - synthesis of 4-acetamidophenylglyoxalmonoxime

SeO<sub>2</sub> (34.18 g, 0.308 mole) and 4-acetamidoacetophenone (49.62 g, 0.28 mole) were suspended in a mixture of dioxane (250 ml) and water (10 ml) and the solution was heated to 100°C for 16 hours. The hot solution was

filtered on a paper filter and the filtrate was evaporated to dryness. The oily residue was then partitioned between  $\text{CHCl}_3$  (400 ml) and a saturated solution of sodium hydrogen-carbonate (200 ml). A precipitation occurs in the aqueous layer. The organic phase was collected and the aqueous layer was filtered  
 5 over a fritted glass. The solid (4-acetamidoglyoxaldehyde) was washed with water and kept for the next step. The aqueous layer was extracted several times with  $\text{CHCl}_3$  and all fractions were collected and evaporated to dryness. The oily residue and the precipitate were put together and suspended in a mixture of water (240 ml) and MeOH (60 ml). Then acetoxime (20.46 g,  
 10 0.28 mole) was added and the pH was adjusted to a range of 3 - 4 with 1 N HCl. The solution was heated to 70 °C for 1 hour, then cooled to 0 °C and the resulting crystals collected. After washing with cold water, then diethylether, drying in a vacuum dessicator over  $\text{P}_2\text{O}_5$  afforded 33.6 g of the desired compound (yield 58%) as yellowish crystals with a purity of 95%, which were  
 15 characterised as follows: MS: m/z (%): 207 ( $[\text{M}+\text{H}]^+$ , 100); UV (MeOH, nm): 241.6, 278.3.

#### Example 91 - synthesis of 3-acetamidophenylglyoxalmonoxime

$\text{SeO}_2$  (36.62 g, 0.33 mole) and 3-acetamidoacetophenone (53.16 g,  
 20 0.30 mole) were suspended in a mixture of dioxane (250 ml) and water (10 ml) and the solution was heated to 100°C for 16 hours. The hot solution was filtered on a paper filter and the filtrate was evaporated to dryness. The oily residue was then suspended in a mixture of water (240 ml) and MeOH (60 ml) and acetoxime (21.93 g, 0.30 mol) was added and the pH was adjusted to a  
 25 range of 3 - 4 with 6 N HCl. The solution was heated to 70 °C for 2 hours and after cooling, the brownish precipitate was filtered and re-crystallised from toluene to afford 45.0 g of the desired compound (yield 72%) with a purity of 98%, which was characterised as follows: MS: m/z (%): 207 ( $[\text{M}+\text{H}]^+$ , 100), 229 ( $[\text{M}+\text{Na}]^+$ , 80); UV (MeOH, nm): 239.2.

30

#### Example 92 - synthesis of 2-amino-4-morpholino-6-(4-acetanilide) pteridine

2,4,5-triamino-6-morpholinopyrimidine dihydrochloride (14.1 g, 50 mmole) was refluxed in MeOH (300 ml) and the compound of example 90

(11.33 g, 55 mmole) in MeOH (150 ml) was added. The mixture was refluxed for 3 hours and after cooling, the yellowish precipitate was filtered, washed with water, methanol, ether and dried at 110°C for 3 hours to afford 15.7 g of the desired product (yield 86%) which, after re-crystallisation from DMF/H<sub>2</sub>O, was obtained with a purity of 97% and characterised as follows: MS: m/z (%): 366 ([M+H]<sup>+</sup>, 100), 753 ([2M+Na]<sup>+</sup>, 15); UV (MeOH, nm): 213, 307, 408.

#### Example 93 – synthesis of 2-amino-4-morpholino-6-(3-acetanilide) pteridine

Repeating the procedure of example 92 but starting from the compound of example 91, the title pteridine derivative (16.1 g) was obtained with a yield of 88% and a purity of 97%, and characterised as follows: MS: m/z (%): 366 ([M+H]<sup>+</sup>, 100), 753 ([2M+Na]<sup>+</sup>, 80); UV (MeOH, nm): 220, 294, 402.

#### Example 94 - synthesis of 2-amino-4-morpholino-6-(4-aminophenyl) pteridine

The crude compound of example 92 (20 mmole) was refluxed in a mixture of MeOH/HCl 6N – 1/1 (400 ml) for 2 hours and cooled in an ice bath. The remaining solid was filtered and the pH was adjusted to 10 with NaOH (10N). The orange precipitate was filtered, washed with water, ethanol, ether and dried in a vacuum dessicator over P<sub>2</sub>O<sub>5</sub> to afford 4.7 g (yield: 73%) of the desired compound as an orange solid with a purity of 98%, which was characterised as follows: MS: m/z (%): 324 ([M+H]<sup>+</sup>, 100); UV (MeOH, nm): 216, 316, 422; <sup>1</sup>H NMR (200 MHz, in DMSO-d<sub>6</sub>, ppm): 3.78 (br s, 4H); 4.33 (br s, 4H); 5.54 (br s, 2H); 6.63 (br s, 2H); 6.67 (d, 2H, J = 8.4 Hz); 7.75 (d, 2H, J = 8.4 Hz); 9.15 (s, 1H).

#### Example 95 - synthesis of 2-amino-4-morpholino-6-(3-aminophenyl) pteridine

Repeating the procedure of example 94 but starting from the compound of example 93, the title pteridine derivative (4.88 g) was obtained with a yield of 74% and a purity of 99.34%, and characterised as follows: MS: m/z (%): 324 ([M+H]<sup>+</sup>, 100); UV (MeOH, nm): 218, 292, 403.

Examples 96 to 102 - coupling reaction between a 2-amino-4-morpholino-6-(4-amino-phenyl) pteridine and a carboxylic acid chloride, sulfonyl chloride or carbamoyl chloride

- 5        The compound of example 94 (162 mg, 0.5 mmole) was suspended in dioxane (10 ml) and triethylamine was added (84  $\mu$ l, 0.6 mmole). Then a suitable carboxylic acid chloride or sulfonyl chloride or carbamoyl chloride (0.55 mmole) was added and the mixture was stirred at room temperature until completion of the reaction. After evaporation to dryness, the crude
- 10        resulting product was purified by flash chromatography on silica gel using a gradient of MeOH (1-5%) in dichloromethane. Yields varied from 50 to 80%. Using this procedure, the following pteridine derivatives were prepared:
- 2-amino-4-morpholino-6-(4-benzoylamino-phenyl) pteridine (example 96),  
purity 98.94%, characterised as follows: MS: m/z (%): 428 ( $[M+H]^+$ , 100),  
15        877 ( $[2M+Na]^+$ , 40); UV (MeOH, nm): 313, 408;
  - 2-amino-4-morpholino-6-(4-phenoxyacetylaminophenyl) pteridine  
(example 97), purity 97.18%, characterised as follows: MS: m/z (%): 458  
( $[M+H]^+$ , 100); UV (MeOH, nm): 305, 407;
  - 2-amino-4-morpholino-6-(4-propionylaminophenyl) pteridine (example 98),  
20        purity 98.86%, characterised as follows: MS: m/z (%): 380 ( $[M+H]^+$ , 100),  
781 ( $[2M+Na]^+$ , 20); UV (MeOH, nm): 213, 307, 408;
  - 2-amino-4-morpholino-6-(4-furoylaminophenyl) pteridine (example 99), ,  
purity 93.96%, characterised as follows: MS: m/z (%): 418 ( $[M+H]^+$ , 100);  
UV (MeOH, nm): 213, 316, 408;
  - 25        - 2-amino-4-morpholino-6-(4-cyclohexanoylamino-phenyl) pteridine  
(example 100), purity 96.91%, characterised as follows: MS: m/z (%): 434  
( $[M+H]^+$ , 100), 889 ( $[2M+Na]^+$ , 10); UV (MeOH, nm): 308, 408;
  - 2-amino-4-morpholino-6-[4-(4-chlorobenzoyl)aminophenyl] pteridine  
(example 101), purity 96.48%, characterised as follows: MS: m/z (%): 462  
30        ( $[M+H]^+$ , 100); UV (MeOH, nm): 313.9, 407; and
  - 2-amino-4-morpholino-6-(4-benzyloxyacetylaminophenyl) pteridine  
(example 102), purity 98.45%, characterised as follows: MS: m/z (%): 472  
( $[M+H]^+$ , 100), 942 ( $[2M+H]^+$ , 5), 965 ( $[2M+Na]^+$ , 10); UV (MeOH, nm):

307, 407.

Examples 103 to 117 - coupling reaction between a 2-amino-4-morpholino-6-aminophenyl pteridine and a carboxylic acid chloride, sulfonyl chloride or carbamoyl chloride

The compound of example 94 or example 95 (162 mg, 0.5 mmole) was suspended in pyridine (10 ml). Then a suitable carboxylic acid chloride or sulfonyl chloride or carbamoyl chloride (0.55 mmole) was added and the mixture was stirred at room temperature until completion of the reaction. After evaporation to dryness, the crude resulting product was purified by flash chromatography on silica gel using a gradient of MeOH (1-5%) in dichloromethane. Yields varied from 20 to 80%. Using this procedure, the following pteridine derivatives were prepared:

- 2-amino-4-morpholino-6-(4-isonicotinoylaminophenyl) pteridine (example 103) was characterised as follows: MS: m/z (%): 429 ([M+H]<sup>+</sup>, 100), 879 ([2M+Na]<sup>+</sup>, 5); UV (MeOH, nm): 213, 313, 407; purity 95.01%;
- 2-amino-4-morpholino-6-(4-naphtoylaminophenyl) pteridine (example 104) was characterised as follows: MS: m/z (%): 478 ([M+H]<sup>+</sup>, 100), 977 ([2M+Na]<sup>+</sup>, 5); UV (MeOH, nm): 213, 313, 407; purity 95.01%;
- 2-amino-4-morpholino-6-(4-methylsulfonylaminophenyl) pteridine (example 105) was characterised as follows: MS: m/z (%): 502 ([M+H]<sup>+</sup>, 100); UV (MeOH, nm): 301, 422; purity 96.81%;
- 2-amino-4-morpholino-6-(4-ethylsuccinylaminophenyl) pteridine (example 106) was characterised as follows: MS: m/z (%): 452 ([M+H]<sup>+</sup>, 100), 925 ([2M+Na]<sup>+</sup>, 15); UV (MeOH, nm): 213, 308, 408; purity: 98.98%;
- 2-amino-4-morpholino-6-[4-(4-methylbenzoate)aminophenyl] pteridine (example 107) was characterised as follows: MS: m/z (%): 486 ([M+H]<sup>+</sup>, 100); UV (MeOH, nm): 236, 315, 407; purity 99.57%;
- 2-amino-4-morpholino-6-(3-benzoylaminophenyl) pteridine (example 108) was characterised as follows: MS: m/z (%): 428 ([M+H]<sup>+</sup>, 100); UV (MeOH, nm): 216, 293, 402; purity: 96.46%;
- 2-amino-4-morpholino-6-(3-benzensulfonylaminophenyl) pteridine (example 109) was characterised as follows: MS: m/z (%): 464 ([M+H]<sup>+</sup>,

- 100); UV (MeOH, nm): 216, 295, 402; purity: 97.49%;
- 2-amino-4-morpholino-6-(3-phenoxyacetylaminophenyl) pteridine (example 110) was characterised as follows: MS: m/z (%): 458 ( $[M+H]^+$ , 100); UV (MeOH, nm): 219, 294, 402; purity 98.96%;
  - 5 - 2-amino-4-morpholino-6-(3-isonicotinoylaminophenyl) pteridine (example 111) was characterised as follows: MS: m/z (%): 429 ( $[M+H]^+$ , 100); UV (MeOH, nm): 216, 294, 402; purity 93.92%;
  - 2-amino-4-morpholino-6-(3-cyclohexanoylaminophenyl) pteridine (example 112) was characterised as follows: MS: m/z (%): 434 ( $[M+H]^+$ , 100); UV (MeOH, nm): 222, 245, 294, 402; purity 99.50%;
  - 10 - 2-amino-4-morpholino-6-[3-(4-methylbenzoate)aminophenyl] pteridine (example 113) was characterised as follows: MS: m/z (%): 486 ( $[M+H]^+$ , 100); UV (MeOH, nm): 218, 238, 294, 402; purity 97.44%;
  - 2-amino-4-morpholino-6-(3-ethylsuccinylaminophenyl) pteridine (example 15 114) was characterised as follows: MS: m/z (%): 452 ( $[M+H]^+$ , 100); UV (MeOH, nm): 222, 245, 294, 402; purity 94.16%;
  - 2-amino-4-morpholino-6-(3-ethylmalonylaminophenyl) pteridine (example 115) was characterised as follows: MS: m/z (%): 438 ( $[M+H]^+$ , 100); UV (MeOH, nm): 220, 244, 294, 402; purity 90.89%;
  - 20 - 2-amino-4-morpholino-6-(3-benzyloxyacetylaminophenyl) pteridine (example 116) was characterised as follows: MS: m/z (%): 472 ( $[M+H]^+$ , 100); UV (MeOH, nm): 216, 243, 294, 402; purity 99.70%; and
  - 2-amino-4-morpholino-6-(3-ethylsulfonylaminophenyl) pteridine (example 117) was characterised as follows: MS: m/z (%): 4716 ( $[M+H]^+$ , 100); UV (MeOH, nm): 218, 295, 402; purity 92.54%.
  - 25

Examples 118 to 121 - coupling reaction between 2-amino-4-morpholino-6-(3-aminophenyl) pteridine and various carboxylic acids

- 30 The compound of example 95 (323 mg, 1 mmole), a carboxylic acid (1.1 mmole) and DIEA (2 mmole) were suspended in dry DMF (10 ml) and benzotriazol-1-yloxy tris(dimethylamino)phosphonium hexafluorophosphate) was added (1.1 mmole). The mixture was stirred at room temperature until completion of the reaction and then diluted with water. The aqueous layer was

extracted with chloroform and the organic layer was evaporated to dryness. The crude resulting product was purified on silica gel using a gradient of MeOH (1-5%) in dichloromethane. Yields varied from 20 to 50%. The following compounds were made according to this procedure:

- 5 - 2-amino-4-morpholino-6-[3-Boc-(L)-phenylalanine-aminophenyl] pteridine (example 118) was characterised as follows: MS: m/z (%): 1141 ( $[2M+H]^+$ , 10), 571 ( $[M+H]^+$ , 100), 515 ( $[M-tBu+H]^+$ , 50), 471 ( $[M-Boc+H]^+$ , 10); UV (MeOH, nm): 213, 245, 294, 402; purity 96.72%;
- 10 - 2-amino-4-morpholino-6-[3-Boc-(D)-phenylalanine-aminophenyl] pteridine (example 119) was characterised as follows: MS: m/z (%): 571 ( $[M+H]^+$ , 100), 515 ( $[M-tBu+H]^+$ , 50), 471 ( $[M-Boc+H]^+$ , 10); UV (MeOH, nm): 213, 245, 294, 402; purity: 95.30%;
- 15 - 2-amino-4-morpholino-6-[3-Boc-(L)-tryptophane-aminophenyl] pteridine (example 120) was characterised as follows: MS: m/z (%): 610 ( $[M+H]^+$ , 100), 554 ( $[M-tBu+H]^+$ , 50), 510 ( $[M-Boc+H]^+$ , 10); UV (MeOH, nm): 220, 291, 402; purity 91.74%; and
- 20 - 2-amino-4-morpholino-6-[3-Boc-(D)-tryptophane-aminophenyl] pteridine (example 121) was characterised as follows: MS: m/z (%): 610 ( $[M+H]^+$ , 100), 554 ( $[M-tBu+H]^+$ , 50), 510 ( $[M-Boc+H]^+$ , 10); UV (MeOH, nm): 220, 291, 402; purity 84.25%.

Example 122 - synthesis of 2-amino-4-morpholino-6-(4-hydroxyphenyl) pteridine

25 To a solution of 2,5,6-triamino-4-morpholino-pyrimidine dihydrochloride salt (5.05 g, 17.8 mmole) in methanol (120 ml) was added 4-hydroxyphenylglyoxalmonoxime (2.95 g, 17.8 mmole). The reaction mixture was refluxed for 3 hours and then cooled down to room temperature. The yellow precipitate was filtered off, washed with water yielding the title compound as a chromatographically pure yellow powder which was further

30 characterised as follows: MS: m/z (%): 671 ( $[2M+Na]^+$ , 5), 325 ( $[M+H]^+$ , 100); UV (MeOH, nm): 213, 302, 411.

Examples 123 to 131 - synthesis of 2-amino-4-morpholino-6-(4-alkoxyphenyl) pteridines

- The following procedure is in accordance with figure 10. To a solution of the compound of example 122 (0.65 mmole, 210 mg) in DMF was added
- 5  $K_2CO_3$  (0.78 mmole, 108 mg) and an appropriate alkyl halide (0.78 mmole). The reaction was stirred overnight at room temperature. The reaction was quenched with water and extracted with  $CH_2Cl_2$ . The organic phase was evaporated in vacuo and the residue purified by flash chromatography (silica, gradient from 1:99 to 3:97  $CH_3OH/CH_2Cl_2$ ) yielding the title compound as a
- 10 yellow powder in yields ranging from 15 to 55. %. The following compounds were made using this procedure:
- 2-amino-4-morpholino-6-(4-ethoxyphenyl) pteridine (example 123) was obtained from iodoethane and characterised as follows: MS: m/z (%): 727 ( $[2M+Na]^+$ , 5), 353 ( $[M+H]^+$ , 100); UV (MeOH, nm): 211, 302, 410;
  - 15 - 2-amino-4-morpholino-6-(4-benzyloxyphenyl) pteridine (example 124) was obtained from benzyl bromide and characterised as follows: MS: m/z (%): 727 ( $[2M+Na]^+$ , 5), 353 ( $[M+H]^+$ , 100); UV (MeOH, nm): 245, 302, 410;
  - 2-amino-4-morpholino-6-(4-(phenethyloxy)-phenyl) pteridine (example 125) was obtained from phenylethyl bromide and characterised as follows:
  - 20 MS: m/z (%): 428 ( $[M+H]^+$ , 100); UV (MeOH, nm): 245, 303, 410;
  - 2-amino-4-morpholino-6-(4-phenoxy-butyronitrile) pteridine (example 126) was obtained from 4-bromobutyronitrile and characterised as follows: MS: m/z (%): 391 ( $[M+H]^+$ , 100);
  - 2-amino-4-morpholino-6-(4-propoxy-phenyl) pteridine (example 127) was
  - 25 obtained from propyl iodide and characterised as follows: MS: m/z (%): 367 ( $[M+H]^+$ , 100);
  - 2-amino-4-morpholino-6-(4-phenoxy-butyric acid ethyl ester) pteridine (example 128) was obtained from ethyl-4-bromobutyrate and characterised as follows: MS: m/z (%): 439 ( $[M+H]^+$ , 100);
  - 30 - 2-amino-4-morpholino-6-(4-phenoxy-acetic acid ethyl ester) pteridine (example 129) was obtained from ethyl bromoacetate and characterised as follows: MS: m/z (%): 843 ( $[2M+H]^+$ , 100), 411 ( $[M+H]^+$ , 100);



- 2-amino-4-morpholino-6-(4-(2-methoxyethoxy)-phenyl) pteridine (example 130) was obtained from 2-bromoethylmethylether and characterised as follows: MS: m/z (%): 787 ( $[2M+H]^+$ , 25), 383 ( $[M+H]^+$ , 100); and
- 2-amino-4-morpholino-6-(4-butoxy-phenyl)-pteridine (example 131) was  
5 obtained from bromobutane and characterised as follows: MS: m/z (%): 381 ( $[M+H]^+$ , 100).

Examples 132 to 172 - synthesis of 2-amino-4-(N-substituted-piperazino)-6-(3,4-dimethoxy-phenyl) pteridines

- 10 To a suspension of the compound of example 6 (1 mmole) in dioxane (20 ml) was added a suitable N-substituted piperazine (1.5 mmole). The suspension was stirred at room temperature for 16 hours. The solvents were evaporated in vacuo yielding crude 2-acetylamino-4-(N-substituted piperazino)-6-(3,4-dimethoxyphenyl) pteridine. Deprotection of the 2-  
15 acetylamino group was achieved by dissolving this crude compound in 20 ml of a 1:1 mixture of methanol and 20%  $K_2CO_3$  in water. The solution was stirred for 16 hours at room temperature. Solvents were evaporated in vacuo and the residue was purified by preparative TLC (silica, using a  $CH_3OH/CH_2Cl_2$  (5:95) mixture as an eluent), affording the following compounds as yellow powders in  
20 yields ranging from 20 to 70%:
- 2-amino-4-(1-(2-methoxyethyl)piperazino)-6-(3,4-dimethoxyphenyl) pteridine (example 132) was obtained from 1-(2-methoxyethyl) piperazine and characterised as follows: MS: m/z (%): 873 ( $[2M+Na]^+$ , 15), 426 ( $[M+H]^+$ , 100);
  - 25 - 2-amino-4-(1-cyclohexylmethyl)piperazino)-6-(3,4-dimethoxyphenyl) pteridine (example 133) was obtained from 1-(cyclohexylmethyl)piperazine and characterised as follows: MS: m/z (%): 949 ( $[2M+Na]^+$ , 5), 464 ( $[M+H]^+$ , 100);
  - 2-amino-4-(1-cyclopentylpiperazino)-6-(3,4-dimethoxyphenyl) pteridine  
30 (example 134) was obtained from 1-cyclopentylpiperazine and characterised as follows: MS: m/z (%): 893 ( $[2M+Na]^+$ , 25), 436 ( $[M+H]^+$ , 100); UV (MeOH, nm): 213, 296, 413;

- 2-amino-4-(1-butylpiperazino)-6-(3,4-dimethoxyphenyl) pteridine (example 135) was obtained from 1-(butyl)piperazine and characterised as follows: MS: m/z (%): 893 ( $[2M+Na]^+$ , 25), 436 ( $[M+H]^+$ , 100]; UV (MeOH, nm): 216, 295, 413;
- 5 - 2-amino-4-(1-isopropylpiperazino)-6-(3,4-dimethoxyphenyl) pteridine (example 136) was obtained from 1-(isopropyl) piperazine and characterised as follows: MS: m/z (%): 841 ( $[2M+Na]^+$ , 20), 410 ( $[M+H]^+$ , 100]; UV (MeOH, nm): 215, 295, 412;
- 2-amino-4-(1-(2-diethylaminoethyl)-piperazino)-6-(3,4-dimethoxyphenyl) pteridine (example 137) was obtained from 1-(2-diethylaminoethyl)-piperazine and characterised as follows: MS: m/z (%): 955 ( $[2M+Na]^+$ , 20), 437 ( $[M+H]^+$ , 100]; UV (MeOH, nm): 216, 297, 413;
- 10 - 2-amino-4-(1-(2-diisopropylaminoethyl)-piperazino)-6-(3,4-dimethoxyphenyl) pteridine (example 138) was obtained from 1-(2-diisopropylaminoethyl)-piperazine and characterised as follows: MS: m/z (%): 495 ( $[M+H]^+$ , 100]; UV (MeOH, nm): 215, 297, 413;
- 15 - 2-amino-4-(1-(2-morpholino-4-yl-ethyl)-piperazino)-6-(3,4-dimethoxyphenyl) pteridine (example 139) was obtained from 1-(2-morpholino-4-yl-ethyl)-piperazine and characterised as follows: MS m/z (%): 481 ( $[M+H]^+$ , 100]; UV (MeOH, nm): 217, 297, 414;
- 20 - 2-amino-4-(4-[2-(piperazin-1-yl)-acetyl]-morpholino)-6-(3,4-dimethoxyphenyl) pteridine (example 140) was obtained from 4-[2-(piperazin-1-yl)-acetyl] morpholine and characterised as follows: MS: m/z (%): 495 ( $[M+H]^+$ , 100]; UV (MeOH, nm): 219, 297, 414;
- 25 - 2-amino-4-(4-[2-(piperazin-1-yl)-acetyl]-pyrrolidino)-6-(3,4-dimethoxyphenyl) pteridine (example 141) was obtained from 4-[2-(piperazin-1-yl)-acetyl] pyrrolidine and characterised as follows: MS: m/z (%): 979 ( $[2M+Na]^+$ , 20), 479 ( $[M+H]^+$ , 100]; UV (MeOH, nm): 219, 307, 411;
- 2-amino-4-(2-[piperazin-1-yl]-acetic acid N-methyl N-phenyl amide)-6-(3,4-dimethoxyphenyl) pteridine (example 142) was obtained from 2-[piperazin-1-yl]-acetic acid N-methyl-N-phenylamide and characterised as follows: MS: m/z (%): 515 ( $[M+H]^+$ , 100]; UV (MeOH, nm): 219, 307, 411;
- 30

- 5        - 2-amino-4-(2-(piperazin-1-yl)-propionic acid ethyl ester)-6-(3,4-dimethoxyphenyl) pteridine (example 143) was obtained from 2-(piperazin-1-yl)-propionic acid ethyl ester (in order to avoid transesterification, a mixture of ethanol and sodium was used for the deprotection of the acetyl group) and characterised as follows: MS:  $m/z$  (%): 468 ( $[M+H]^+$ , 100); UV (MeOH, nm) : 216, 297, 413;
- 10       - 2-amino-4-(3-(piperazin-1-yl)-propionic acid ethyl ester)-6-(3,4-dimethoxyphenyl) pteridine (example 144) was obtained from 3-(piperazin-1-yl)-propionic acid ethyl ester (in order to avoid transesterification, a mixture of ethanol and sodium (15 equivalents) was used for the deprotection of the acetyl group) and characterised as follows: MS:  $m/z$  (%): 957 ( $[2M+Na]^+$ , 10), 468 ( $[M+H]^+$ , 100); UV (MeOH, nm) : 216, 296, 412;
- 15       - 2-amino-4-(2-(piperazin-1-yl)-acetic acid ethyl ester)-6-(3,4-dimethoxyphenyl) pteridine (example 145) was obtained from 3-(piperazin-1-yl)-propionic acid ethyl ester (in order to avoid transesterification, a mixture of ethanol and sodium (15 equivalents) was used for the deprotection of the acetyl group) and characterised as follows: MS:  $m/z$  (%): 454 ( $[M+H]^+$ , 100); UV (MeOH, nm) : 216, 297, 413;
- 20       - 2-amino-4-(1-(3-methyl-benzyl)piperazinyl)-6-(3,4-dimethoxyphenyl) pteridine (example 146) was obtained from 1-(3-methylbenzyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 965 ( $[2M+Na]^+$ , 10), 472 ( $[M+H]^+$ , 100);
- 25       - 2-amino-4-[(2,6-dichlorobenzyl)piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 147) was obtained from 1-(2,6-dichloro-benzyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 526 ( $[M+H]^+$ , 100);
- 2-amino-4-[(4-fluorobenzyl)piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 148) was obtained from 1-(4-fluorobenzyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 476 ( $[M+H]^+$ , 100);
- 30       - 2-amino-4-[(4-chlorobenzyl)piperazin-1-yl]-6-(3,4-dimethoxyphenyl) pteridine (example 149) was obtained from 1-(4-chloro-benzyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 492 ( $[M+H]^+$ , 100);

- 2-amino-4-((4-methylbenzyl)piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 150) was obtained from 1-(4-methyl-benzyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 472 ( $[M+H]^+$ , 100);
- 2-amino-4-((2-fluorobenzyl)piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 151) was obtained from 1-(2-fluorobenzyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 476 ( $[M+H]^+$ , 100);
- 2-amino-4-((3,4-dichlorobenzyl)piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 152) was obtained from 1-(3,4-dichlorobenzyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 526 ( $[M+H]^+$ , 100);
- 2-amino-4-(piperonyl-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 153) was obtained from 1-piperonyl-piperazine and characterised as follows: MS:  $m/z$  (%): 502 ( $[M+H]^+$ , 100);
- 2-amino-4-((4-*tert*-butylbenzyl)piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 154) was obtained from 1-(4-*tert*-butyl-benzyl)-piperazine) and characterised as follows: MS:  $m/z$  (%): 514 ( $[M+H]^+$ , 100);
- 2-amino-4-((4-pyridyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 155) was obtained from 1-(4-pyridyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 445 ( $[M+H]^+$ , 100); UV (MeOH, nm): 213, 289, 411;
- 2-amino-4-((2-pyridyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 156) was obtained from 1-(2-pyridyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 911 ( $[2M+Na]^+$ , 60), 889 ( $[2M+H]^+$ , 60), 445 ( $[M+H]^+$ , 100); UV (MeOH, nm): 215, 247, 297, 413;
- 2-amino-4-((2-pyrimidinyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 157) was obtained from 1-(2-pyrimidinyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 446 ( $[M+H]^+$ , 100); UV (MeOH, nm): 216, 244, 297, 413;
- 2-amino-4-((3-methoxyphenyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 158) was obtained from 1-(3-methoxyphenyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 969 ( $[2M+Na]^+$ , 15), 446 ( $[M+H]^+$ , 100); UV (MeOH, nm): 215, 295, 413;

- 2-amino-4-(1-(3-phenylpropyl)-piperazine)-6-(3,4-dimethoxyphenyl) pteridine (example 159) was obtained from 1-(3-phenylpropyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 486 ( $[M+H]^+$ , 100); UV (MeOH, nm): 217, 296, 413;
- 5    - 2-amino-4-((3,4-dichlorophenyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 160) was obtained from 1-(3,4-dichlorophenyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 512 ( $[M+H]^+$ , 100); UV (MeOH, nm): 213, 263, 295, 412;
- 2-amino-4-((3-dichlorophenyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 161) was obtained from 1-(3-dichlorophenyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 478 ( $[M+H]^+$ , 100); UV (MeOH, nm): 213, 257, 296, 413;
- 10    - 2-amino-4-((1-phenylethyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 162) was obtained from 1-(1-phenylethyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 965 ( $[2M+Na]^+$ , 10), 472 ( $[M+H]^+$ , 100); UV (MeOH, nm): 216, 298, 413;
- 15    - 2-amino-4-((2-(1-pyrrolyl)-ethyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 163) was obtained from 1-[2-(1-(pyrrolyl)-ethyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 461 ( $[M+H]^+$ , 100); UV (MeOH, nm): 216, 297, 413;
- 20    - 2-amino-4-((2-phenoxyethyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 164) was obtained from 1-(2-phenoxyethyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 488 ( $[M+H]^+$ , 100); UV (MeOH, nm): 216, 297, 413;
- 25    - 2-amino-4-(1-(2-imidazol-1-yl-ethyl)-piperazine)-6-(3,4-dimethoxyphenyl) pteridine (example 165) was obtained from 1-(2-imidazol-1-yl-ethyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 945 ( $[2M+Na]^+$ , 10), 462 ( $[M+H]^+$ , 100); UV (MeOH, nm): 216, 297, 413;
- 2-amino-4-((3-pyridyl)-methyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 166) was obtained from 1-(3-pyridyl)-methyl-piperazine and characterised as follows: MS:  $m/z$  (%): 939 ( $[2M+Na]^+$ , 15), 459 ( $[M+H]^+$ , 100); UV (MeOH, nm): 215, 297, 413;
- 30

- 2-amino-4-((4-pyridyl)-methyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 167) was obtained from 1-(4-pyridyl)-methyl-piperazine and characterised as follows: MS:  $m/z$  (%): 939 ( $[2M+Na]^+$ , 15), 459 ( $[M+H]^+$ , 100); UV (MeOH, nm): 218, 297, 414;
- 5 - 2-amino-4-((1-naphtylmethyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 168) was obtained from 1-(1-naphtylmethyl)-piperazine and characterised as follows: MS:  $m/z$  (%): 508 ( $[M+H]^+$ , 100); UV (MeOH, nm): 223, 297, 413;
- 2-amino-4-(*N*-phenethylpiperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine  
10 (example 169) was obtained from *N*-phenethylpiperazine and characterised as follows: MS:  $m/z$  (%): 965 ( $[2M+Na]^+$ , 10), 472 ( $[M+H]^+$ , 100); UV (MeOH, nm): 215, 297, 413;
- 2-amino-4-((2-methoxyphenyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 170) was obtained from 1-(2-methoxyphenyl)-  
15 piperazine and characterised as follows: MS:  $m/z$  (%): 474 ( $[M+H]^+$ , 100); UV (MeOH, nm): 213, 295, 413;
- 2-amino-4-((4-methoxyphenyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 171) was obtained from 1-(4-methoxyphenyl)-  
20 piperazine and characterised as follows: MS:  $m/z$  (%): 474 ( $[M+H]^+$ , 100); UV (MeOH, nm): 212, 296, 413; and
- 2-amino-4-((4-chlorophenyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 172) was obtained from 1-(4-chlorophenyl)piperazine and characterised as follows: MS:  $m/z$  (%): 478 ( $[M+H]^+$ , 100); UV (MeOH, nm): 258, 296, 413.

25

Examples 173 to 190 - synthesis of 2-amino-6-(3,4-dimethoxyphenyl)-4-(substituted piperazin-1-yl) pteridines

The following procedure is in accordance with the scheme shown in figure 3. A mixture of the compound of example 4 (299 mg, 1.0 mmole),  
30 hexamethyldisilazane (1 ml, 4.7 mmole), a substituted piperazine ( 4.0 mmole), p-toluenesulfonic acid (20 mg, 0.1 mmole) and ammonium sulfate (20 mg, 0.15 mmole) in toluene (4 ml) was refluxed for 48 hours (the reaction mixture became clear when the reaction was finished). After removing the

solvents under reduced pressure. the residue was purified by flash chromatography over silica (CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> 1:20 to 1:30) reaching the desired following compounds as yellow solids in yields indicated below:

- 2-amino-6-(3,4-dimethoxyphenyl)-4-(4-(3-propionitril)-piperazin-1-yl) pteridine (example 173) was obtained from 3-(1-piperazinyl)-propionitrile in 43 % yield and characterized as follows: R<sub>f</sub> = 0.50 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 215, 297, 412; MS (m/z): 421 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-(4-(2-(1,3)-dioxolan-2-yl-ethyl)-piperazin-1-yl) pteridine (example 174) was obtained from 2-[2-(piperazin-1-yl)-ethyl]-1,3-dioxolane in 55 % yield and characterized as follows: R<sub>f</sub> = 0.49 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 215, 295, 412; MS (m/z): 468 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-(4-(2-ethoxyethyl)-piperazin-1-yl) pteridine (example 175) was obtained from 1-(2-ethoxyethyl)-piperazine in 35 % yield and characterized as follows: R<sub>f</sub> = 0.33 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 213, 295, 412; MS (m/z): 440 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-(pent-3-yl-piperazin-1-yl) pteridine (example 176) was obtained from 1-(3-pentyl)-piperazine in 22 % yield and characterized as follows: R<sub>f</sub> = 0.43 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 212, 293, 412; MS (m/z): 438.2 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-(1-pentyl-piperazin-1-yl) pteridine (example 177) was obtained from 1-(1-pentyl)-piperazine in 22 % yield and characterized as follows: R<sub>f</sub> = 0.54 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 212, 294, 412; MS (m/z): 438 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-(1-isobutyl-piperazin-1-yl) pteridine (example 178) was obtained from 1-isobutylpiperazine in 26 % yield and characterized as follows: R<sub>f</sub> = 0.42 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 213, 294, 412; MS (m/z): 424 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-((tetrahydrofurfuryl)-piperazin-1-yl) pteridine (example 179) was obtained from 1-tetrahydrofurfuryl-piperazine in 31 % yield and characterized as follows: R<sub>f</sub> = 0.37 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 215, 295, 412; MS (m/z): 453 ([M+H]<sup>+</sup>, 100);

- 2-amino-6-(3,4-dimethoxyphenyl)-4-(1,3-dioxolan-2-yl-methylpiperazin-1-yl) pteridine (example 180) was obtained from 2-(piperazin-1-yl-methyl)-1,3-dioxolane in 65 % yield and characterized as follows:  $R_f = 0.46$  (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 217, 297, 413; MS (m/z): 454 ([M+H]<sup>+</sup>, 100);
- 2-amino-4-((3,5-dichlorophenyl)-piperazin-1-yl)-6-(3,4-dimethoxyphenyl) pteridine (example 181) was obtained from 1-(3,5-dichlorophenyl)-piperazine in 83 % yield and characterised as follows:  $R_f = 0.70$  (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 216, 263, 296, 413; MS (m/z): 512.2 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-((4-fluorophenyl)-piperazin-1-yl) pteridine (example 182) was obtained from 1-(4-fluororophenyl)-piperazine in 20 % yield and characterised as follows:  $R_f = 0.53$  (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 212, 296, 413; MS (m/z): 462.2 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-((3-trifluoromethylphenyl)-piperazin-1-yl) pteridine (example 183) was obtained from 1-(3-trifluororomethylphenyl)-piperazine in 71 % yield and characterised as follows:  $R_f = 0.58$  (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 212, 257, 296, 413; MS (m/z): 512.2 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-((3,4-dimethylphenyl)-piperazin-1-yl) pteridine (example 184) was obtained from 1-(3,4-dimethylphenyl)-piperazine in 48 % yield and characterised as follows:  $R_f = 0.58$  (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 243, 296, 413; MS (m/z): 472.3 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-((3-methylphenyl)-piperazin-1-yl) pteridine (example 185) was obtained from 1-(3-methylphenyl)-piperazine in 59 % yield and characterised as follows:  $R_f = 0.47$  (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 218, 244, 297, 413; MS (m/z): 458.2 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-((4-methylphenyl)-piperazin-1-yl) pteridine (example 186) was obtained from 1-(4-methylphenyl)-piperazine in 59 % yield and characterised as follows:  $R_f = 0.50$  (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 213, 242, 296, 413; MS (m/z): 458.2 ([M+H]<sup>+</sup>, 100);



- 2-amino-6-(3,4-dimethoxyphenyl)-4-((2-pyridyl)methyl-piperazin-1-yl) pteridine (example 187) was obtained from 1-((2-pyridyl)-methyl)-piperazine in 27 % yield and characterised as follows: Rf = 0.38 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 216, 297, 413; MS (m/z): 459.2 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-(thiazol-2-yl)-piperazin-1-yl) pteridine (example 188) was obtained from 1-thiazol-2-yl-piperazine in 11 % yield and characterised as follows: Rf = 0.55 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 213, 294, 413; MS (m/z): 451.2 ([M+H]<sup>+</sup>, 100);
- 2-amino-6-(3,4-dimethoxyphenyl)-4-(1-(1-methyl-piperidin-3-yl-methyl)-piperazin-1-yl) pteridine (example 189) was obtained from 1-(1-methyl-piperidin-3-yl-methyl)-piperazine in 48 % yield and characterised as follows: MS (m/z): 479 ([M+H]<sup>+</sup>, 100); UV (MeOH/H<sub>2</sub>O, nm): 217, 266, 297, 412; and
- 2-amino-6-(3,4-dimethoxyphenyl)-4-((4-trifluoromethylphenyl)-piperazin-1-yl) pteridine (example 190) was obtained from 1-(4-trifluoromethylphenyl)-piperazine in 48 % yield and characterised as follows: Rf = 0.55 (MeOH/CH<sub>2</sub>Cl<sub>2</sub>=1/9); UV (MeOH/H<sub>2</sub>O, nm): 212, 266, 294, 412; MS (m/z): 512.2 ([M+H]<sup>+</sup>, 100).

Examples 191 to 194 - synthesis of 2-amino-6-(3,4-dimethoxyphenyl)-4-(substituted piperazin-1-yl) pteridine trihydrochloride salts

A mixture of the compound of example 4 (299 mg, 1.0 mmole), hexamethyldisilazane (1 ml, 4.7 mmole), a substituted piperazine ( 4.0 mmole), p-toluenesulfonic acid (20 mg, 0.1 mmole) and ammonium sulfate (20 mg, 0.15 mmole) in toluene (4 ml) was refluxed for 48 hours (the reaction mixture became clear when the reaction was finished). After removing the solvents under reduced pressure, the residue was purified by flash chromatography over silica (CH<sub>3</sub>OH/CH<sub>2</sub>Cl<sub>2</sub> 1:20 to 1:30). To a solution of the resulting free pteridine base in methanol (20 ml), 1.25 M HCl in MeOH (4 ml, 5.0 mmole) was slowly added. The mixture was stirred at room temperature for one hour. The precipitate (which is the trihydrochloride salt of the free

pteridine base) was filtered off and washed with methanol. Drying in the vacuum over  $P_2O_5$  yielded the corresponding hydrochloride salt as a yellow solid. The following salts were made according to this procedure, with yields indicated below:

- 5 - 2-amino-6-(3,4-dimethoxyphenyl)-4-(4-(2-dimethylaminoethyl)-piperazin-1-yl)-pteridine trihydrochloride salt (example 191) obtained in 58% yield was characterised as follows:  $R_f = 0.20$  (MeOH/Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub>=4/2/100); UV (MeOH/H<sub>2</sub>O, nm): 215, 297, 412; MS (m/z): 439 ([M-3HCl+H]<sup>+</sup>, 100);
- 10 - 2-amino-6-(3,4-dimethoxyphenyl)-4-(4-(3-dimethylaminopropyl)-piperazin-1-yl) pteridine trihydrochloride salt (example 192) obtained in 57 % yield was characterised as follows:  $R_f = 0.25$  (MeOH/Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub>=4/2/100); UV (MeOH/H<sub>2</sub>O, nm): 214, 296, 412; MS (m/z): 453 ([M-3HCl+H]<sup>+</sup>, 100);
- 15 - 2-amino-6-(3,4-dimethoxyphenyl)-4-(4-(2-dipropylaminoethyl)-piperazin-1-yl) pteridine trihydrochloride salt (example 193) obtained in 52 % yield was characterised as follows:  $R_f = 0.40$  (MeOH/Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub>=4/2/100); UV (MeOH/H<sub>2</sub>O, nm): 216, 297, 413; MS (m/z): 495 ([M-3HCl+H]<sup>+</sup>, 100); and
- 20 - 2-amino-6-(3,4-dimethoxyphenyl)-4-(4-(2-piperidin-1-yl-ethyl)-piperazin-1-yl) pteridine trihydrochloride salt (example 194) obtained in 44 % yield was characterised as follows:  $R_f = 0.35$  (MeOH/Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub>=4/2/100); UV (MeOH/H<sub>2</sub>O, nm): 216, 297, 412; MS (m/z): 479 ([M-3HCl+H]<sup>+</sup>, 100).

#### Example 195 – mixed lymphocyte reaction assay

25 Pteridine derivatives were first dissolved (10mM) in dimethylsulfoxide (hereinafter referred as DMSO) and further diluted in culture medium before use for the following *in vitro* experiments. The commercially available culture medium consisted of RPMI-1640 + 10% foetal calf serum (FCS). Some pteridine derivatives described in the previous examples (as indicated in table 1) were tested in the following mixed lymphocyte reaction (MLR) assay.

30 Peripheral blood mononuclear cells (hereinafter referred as PBMC) were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (Nycomed, Maorstua, Norway). Allogeneic PBMC or Epstein-Barr Virus-transformed human B cells [commercially available under the trade name RPMI1788 (ATCC name CCL156)] which

strongly express B7-1 and B7-2 antigens were used as stimulator cells after irradiation with 30 Gy. MLR was performed in triplicate wells. After 5 days incubation at 37°C, 1 µCi [<sup>3</sup>H]-thymidine was added to each cup. After a further 16 hours incubation, cells were harvested and counted in a β-counter.

- 5 Inhibition of proliferation by a compound (drug) described in some of the previous examples was counted using the formula:

$$\% \text{ inhibition} = \frac{(\text{cpm} + \text{drugs}) - (\text{cpm cult. med})}{(\text{cpm} - \text{drugs}) - (\text{OD cult. med})} \times 100$$

- wherein cpm is the thymidine count per minute. The MLR assay is regarded by those skilled in the art as an *in vitro* analogue of the transplant rejection since it is based on the recognition of allogeneic major histocompatibility antigens on the stimulator leukocytes, by responding lymphocytes.

- Table 1 below shows the IC<sub>50</sub> values for various pteridine derivatives of this invention in the MLR test. The IC<sub>50</sub> value represents the lowest concentration of said pteridine derivative (expressed in µmole/l) that resulted in a 50 % suppression of the MLR.

TABLE 1

Example n°	MLR	Example n°	MLR	Example n°	MLR
8	0.9	29	< 0.1	28	< 0.1
9	1.0	30	0.1	48	0.3
10	0.1	31	3.0	49	0.4
11	0.4	32	0.4	50	0.065
12	0.3	36	< 0.1	51	0.3
13	0.1	37	< 0.1	55	5.1
14	0.2	38	0.3	56	2.8
15	0.1	39	0.7	57	7.6
16	0.8	40	0.6	58	1.9
17	0.1	41	0.5	60	4.1
18	0.3	42	0.4	62	10.0
19	2.4	43	0.2	63	10.0

Example n°	MLR	Example n°	MLR	Example n°	MLR
20	0.5	44	0.2	64	0.4
21	6.0	45	< 0.1	65	0.9
26	0.5	46	0.6	66	0.8
27	< 0.1	47	0.3	79	4.1
80	4.7	82	3.2	87	8.4
88	0.8	92	8.6	93	6.4
94	1.1	95	4.1	96	2.1
97	2.5	98	6.5	99	7.8
100	4.1	103	3.9	104	4.0
108	4.9	109	0.8	110	10.0
111	10.0	112	2.9	116	4.1
117	3.3	118	3.9	119	4.1
120	0.9	121	0.6	122	4.3
123	3.7	127	6.8	130	5.4
132	0.8	133	3.9	134	0.9
135	0.8	136	4.9	137	9.1
138	7.4	139	2.5	140	1.5
141	0.3	142	< 0.1	143	1.2
146	1.4	147	7.6	148	6.0
151	0.7	154	4.3	155	3.5
156	0.2	157	0.2	158	0.3
159	0.5	160	1.0	161	0.6
162	1.2	163	0.2	164	0.1
165	3.6	166	0.6	167	0.5
168	0.8	169	0.11	170	3.8
171	0.4	172	0.8	145	0.6
173	0.5	174	2.3	175	2.9
176	5.3	177	3.8	178	1.6
179	0.8	181	3.4	182	0.1

Example n°	MLR	Example n°	MLR	Example n°	MLR
183	0.7	184	0.5	185	0.4
186	0.3	187	0.8	188	1.3
189	7.9	193	8.2	194	7.7

Table 1 (end)

Example 196 - TNF- $\alpha$  assay

Peripheral blood mononuclear cells (herein referred as PBMC), in response to stimulation by lipopolysaccharide (hereinafter LPS), a gram-negative bacterial endotoxin, produce various chemokines, in particular human TNF- $\alpha$ . Inhibition of the activation of PBMC can therefore be measured by the level of suppression of the production of TNF- $\alpha$  by PBMC in response to stimulation by LPS.

Such inhibition measurement was performed as follows: PBMC were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (commercially available from Nycomed, Norway). LPS was then added to the PMBC suspension in complete medium ( $10^6$  cells /ml) at a final concentration of 1  $\mu$ g/ml. The pteridine derivative to be tested was added at different concentrations (0.1  $\mu$ M, 1  $\mu$ M and 10  $\mu$ M) and the cells were incubated at 37°C for 72 hours in 5% CO<sub>2</sub>. The supernatants were collected, then TNF- $\alpha$  concentrations were measured with an anti-TNF- $\alpha$  antibody in a sandwich ELISA (Duo Set ELISA human TNF $\alpha$ , commercially available from R&D Systems, United Kingdom). The colorimetric reading of the ELISA was measured by a Multiskan RC plate reader (commercially available from ThermoLabsystems, Finland) at 450 nm (reference wavelength: 690 nm). Data analysis was performed with Ascent software 2.6. (also from ThermoLabsystems, Finland): a standard curve (recombinant human TNF $\alpha$ ) was drawn and the amount (pg/ml) of each sample on the standard curve was determined.

The % suppression of human TNF $\alpha$  production by the pteridine derivatives of the invention (drugs) was calculated using the formula:

$$\% \text{ suppression} = \frac{\text{pg/ml in drugs} - \text{pg/ml in cult. med.}}{(\text{pg/ml in cult. med.} + \text{LPS}) - \text{pg/ml cult. med.}}$$

Table 2 below shows the IC<sub>50</sub> values (expressed in  $\mu\text{M}$ ) of the tested pteridine derivatives in the TNF- $\alpha$  assay.

TABLE 2

Example n°	TNF- $\alpha$	Example n°	TNF- $\alpha$	Example n°	TNF- $\alpha$
8	0.4	29	0.7	48	0.08
9	0.14	30	0.3	49	0.07
10	0.07	31	0.5	50	0.02
11	0.4	32	0.4	51	0.04
12	0.2	36	0.4	56	3.7
13	0.08	37	0.1	60	2.5
14	0.06	38	0.2	62	7.6
15	0.1	39	0.8	64	0.05
16	0.55	40	0.7	65	0.095
17	0.08	41	0.9	66	0.3
18	0.09	42	0.1	79	9.8
19	0.06	43	0.7	80	10.0
20	0.03	44	0.7	82	9.3
26	0.5	45	0.4	92	8.4
27	0.5	46	0.2	94	2.5
28	0.5	47	0.6	97	4.9
103	4.4	108	4.3	109	0.5
111	7.3	112	5.0	114	5.5
116	7.7	117	10.0	118	2.9
122	2.1	123	10.0	127	10.0
128	0.9	129	0.6	130	7.2
132	0.6	133	0.8		
134	0.4	136	0.9	137	2.6

Example n'	TNF- $\alpha$	Example n'	TNF- $\alpha$	Example n'	TNF- $\alpha$
138	3.3	139	0.8	140	1.4
141	1.9	142	0.05	143	0.33
144	0.07	145	0.27	146	0.8
		147	0.7	149	0.4
150	1.3	151	0.2	153	0.8
154	0.7	155	4.4	156	0.2
157	0.1	158	0.3	159	0.3
160	0.3	161	0.1	162	0.3
163	0.1	164	0.03	165	0.9
166	0.4	167	0.3	168	0.7
169	0.02	170	0.42	171	0.06
172	0.4	173	0.5	174	0.2
175	0.3	176	0.8	177	0.3
178	0.09	179	0.3	180	0.4
181	0.5	182	0.05	184	0.11
186	< 0.01	187	0.09	188	0.15
189	1.8	190	1.4	191	8.8
192	4.8	193	1.2	194	0.7

Table 2 (end)

Example 197 - protection against a lethal dose of TNF- $\alpha$ 

- 5 A model of TNF- $\alpha$  induced shock in C57BL/6 male mice was performed as follows. Five animals from the control group received an intravenous administration of a lethal dose of TNF- $\alpha$  (10  $\mu$ g) in the tail. Ten animals from the test group received three intraperitoneous injections of the pteridine derivative of example 17 (20 mg/kg/day) respectively 48 hours, 24 hours and immediately
- 10 before an intravenous injection of TNF- $\alpha$  (10  $\mu$ g).

Body temperature, a clinical sign of TNF-induced shock, was followed for 40 hours in control mice and in mice receiving the pteridine derivative of example 17:

the body temperature of control mice dropped significantly when compared to mice receiving the test compound of example 17.

Furthermore, all five mice from the control group died within 40 hours, the survival rate (80%) of mice that received the pteridine derivative of example 17 in addition to the TNF- $\alpha$  dose (10  $\mu$ g) was quite substantial.

#### Example 198 – inhibition of the metastasis of melanoma cells in mice

C57BL/6 mice were injected with  $1.5 \times 10^6$  B16BL/6 melanomasarcoma cells subcutaneously in the foot path and were divided, three days later, into 4 groups:

- a control 1 group of 6 mice receiving only vehicle 3 times a week;
- a control 2 group of 5 mice receiving a lethal dose of TNF (15  $\mu$ g, subcutaneously) 3 times a week;
- a group 3 receiving the pteridine derivative of example 17 alone 3 times a week at 20mg/kg
- a group 4 of 7 mice receiving the pteridine derivative of example 17 in an amount of 20 mg/kg intraperitenously on days 3, 4, and 5 and also receiving TNF (15  $\mu$ g, subcutaneously) on day 5. This combined treatment with TNF and the pteridine derivative of example 17 was continued for 2 weeks.

Tumor size data (tumor size was measured as the largest diameter multiplied by the smallest diameter) show that the combined treatment in group 4 leads to a significant reduction of tumor size (120 mm<sup>2</sup>) when compared to the control group 1 (tumor size: 440 mm<sup>2</sup>). Reduction of tumor size is also true in mice of group 3, although to a lesser extent (198 mm<sup>2</sup>).

All mice of control group 2 died within the very first days of treatment, whereas mortality was 3/7 in mice of group 4.

At the end of experiment, it was looked macroscopically at black metastasis in inguinal and/or para-aortic lymphoneuds in all tumor bearing groups of mice. The proportions of mice having metastasis were:

- 4/5 mice in control group 1,



- 0/4 mice in group 4, and
- 2/4 in group 3.

#### Example 199 – model of rheumatoid arthritis

5 Collagen type II (hereinafter referred as CII) induced experimental model of rheumatoid arthritis (hereinafter referred as RA) in DBA mice is widely accepted as the most relevant and predictive preclinical model for RA. In this model, DBA mice are immunized with CII, the collagen type mainly present in the joint structures, together with complete Freund Adjuvant in their tail. 2 to 3 weeks  
10 later, several of the immunized mice start to develop arthritis in the four footpaths. In order to further worsen the disease, mice are given a second CII boost at three weeks after the first immunisation, this time however in a footpath. Because the immune system is already immunised in these mice, this rapidly provokes a severe swelling of the injected footpath (named Delayed Type Hypersensitivity or  
15 DTH) which can be used as a measurement for T-cell activation. Within a few days after the booster, almost all untreated animals start developing symptoms of arthritis. RA development is scored from 0 to 16 (16 being severe clinical arthritis in all four footpaths). At the end of the study (3 weeks after the CII boost) antibody formation was determined against CII and histology performed on the  
20 footpaths.

The efficiency of the pteridine derivative of example 17 (administered in an amount of 20 mg/kg/day, started one day before the CII boost) was explored in this CII-model. All such treated animals developed significantly less severe rheumatoid arthritis (clinical scores ranging from 2 to 4), as compared to  
25 untreated control mice (clinical scores ranging from 6 to 12) and also compared to mice treated with methotrexate (clinical scores ranging from 2 to 7), the most effective compound for the treatment of RA to date.

Additionally, increasing the dose of the pteridine derivative of example 17 up to 40 mg/kg/day has no mortality or cytotoxic effect on mice *in vivo*, whereas  
30 increasing the dose of methotrexate (10 mg/kg/day, 3 times a week) leads to death of all animals.

#### Example 200 – model of protection against septic shock

As a control group, 4 sham treated (saline injection) C3H mice being injected intraperitoneously with 100 µg lipopolysaccharide (hereinafter LPS) per mouse, all died within 1 to 3 days after injection. However, when four C3H mice being injected intraperitoneously with 100 µg lipopolysaccharide (hereinafter LPS) per mouse were treated during 2 days with the pteridine derivative of example 17 (one first intraperitoneous injection of 20 mg/kg/day at the time of injection, and a second injection 24 hours later), all mice were protected from acute shock related mortality.

10

#### Example 201 - IL-1 β assay

Peripheral blood mononuclear cells (herein referred as PBMC), in response to stimulation by lipopolysaccharide (LPS), a gram-negative bacterial endotoxin, produce various chemokines, in particular human IL-1 β. Inhibition of the activation of PBMC can therefore be measured by the level of suppression of the production of IL-1 β by PBMC in response to stimulation by LPS.

15

Such inhibition measurement was performed as follows: PBMC were isolated from heparinized peripheral blood by density gradient centrifugation over Lymphoprep (commercially available from Nycomed, Norway). LPS was then added to the PMBC suspension in complete medium ( $10^6$  cells /ml) at a final concentration of 1 µg/ml. The pteridine derivative to be tested was added at different concentrations (0.1 µM, 1 µM and 10 µM) and the cells were incubated at 37°C for 72 hours in 5% CO<sub>2</sub>. The supernatants were collected, then IL-1 β concentrations were measured with an anti-IL-1 β antibody in a sandwich ELISA.

20

The colorimetric reading of the ELISA was measured by a Multiskan RC plate reader (commercially available from ThermoLabsystems, Finland) at 450 nm (reference wavelength: 690 nm). Data analysis was performed with Ascent software 2.6. (also from ThermoLabsystems, Finland) : a standard curve (recombinant human IL-1 β) was drawn and the amount (pg/ml) of each sample on the standard curve was determined.

25  
30

The % suppression of human IL-1  $\beta$  by the pteridine derivatives (drugs) of this invention was calculated using the formula:

$$\% \text{ suppression} = \frac{\text{pg/ml in drugs} - \text{pg/ml in cult. med.}}{(\text{pg/ml in cult. med.} + \text{LPS}) - \text{pg/ml cult. med.}}$$

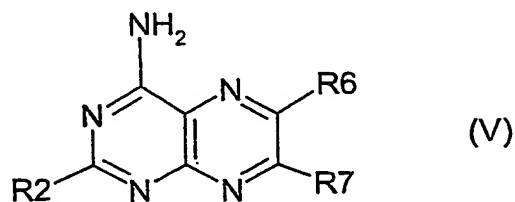
- 5 Table 3 below shows the IC<sub>50</sub> values (expressed in  $\mu\text{M}$ ) of the tested pteridine derivatives in the IL-1  $\beta$  assay.

TABLE 3

Example n°	IL-1 $\beta$	Example n°	IL-1 $\beta$	Example n°	IL-1 $\beta$
64	4.4	65	3.5	132	10.0
133	4.2	134	5.0	135	7.5
136	3.7	142	5.3	144	1.2
146	4.7	149	1.2	151	0.6
156	5.0	157	4.3	158	7.2
159	4.9	160	3.5	161	3.7
162	3.6	163	2.0	164	2.2
167	2.3	169	5.4	171	3.2
172	3.8	176	4.4	177	3.7
182	5.1	183	4.6	184	5.0
186	4.9	188	4.0	190	4.4

CLAIMS

1. A pteridine derivative having the general formula (V):



wherein:

- R<sub>2</sub> is selected from the group consisting of nitrogen-containing heterocyclic radicals other than morpholinyl and piperazinyl, said nitrogen-containing heterocyclic radicals being attached to the pteridine ring by means of a nitrogen atom; benzylamino; phenylethylamino; heterocyclic-substituted alkylamino; aryloxy; arylthio; arylsulfonyl; arylalkyloxy; arylalkylthio; C<sub>1-7</sub> alkylsulfonyl; heterocyclic-substituted alkyloxy; heterocyclic-substituted alkylthio;
- R<sub>6</sub> and R<sub>7</sub> are independently selected from the group consisting of hydrogen; halogen; C<sub>1-7</sub> alkyl; C<sub>2-7</sub> alkenyl; C<sub>2-7</sub> alkynyl; halo C<sub>1-7</sub> alkyl; carboxy C<sub>1-7</sub> alkyl; C<sub>1-7</sub> alkylsulfonyl; carboxyaryl; C<sub>1-7</sub> alkoxy; C<sub>3-10</sub> cycloalkoxy; aryloxy; arylalkyloxy; oxyheterocyclic; heterocyclic-substituted alkyloxy; C<sub>1-7</sub> alkylthio; thio C<sub>3-10</sub> cycloalkyl; arylthio; arylsulfonyl; thioheterocyclic; arylalkylthio; heterocyclic-substituted alkylthio; hydroxylamino; mercaptoamino; acylamino; thioacylamino; alkoxyamino; thioalkylamino; acetal; thio-acetal; carboxylic acid; carboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; thiocarboxylic acid; thiocarboxylic acid esters, thioesters, halides, anhydrides, amides and thioamides; hydroxyl; sulfhydryl; nitro; cyano; carbamoyl; thiocarbamoyl; ureido; thio-ureido; amino; alkylamino; cycloalkylamino; alkenylamino; cycloalkenylamino; alkynylamino; arylamino; arylalkylamino; hydroxyalkylamino; mercaptoalkylamino; heterocyclic amino; heterocyclic-substituted alkylamino; oximino; alkyloximino; hydrazino; alkylhydrazino; phenylhydrazino; cysteinyl acid, esters, thioesters, halides, anhydrides, amides and thioamides thereof; aryl optionally substituted with one or

more substituents independently selected from the group consisting of  
 halogen, C<sub>1-7</sub> alkyl, C<sub>2-7</sub> alkenyl, C<sub>2-7</sub> alkynyl, halo C<sub>1-7</sub> alkyl, nitro, hydroxyl,  
 sulfhydryl, amino, C<sub>1-7</sub> alkoxy, C<sub>3-10</sub> cycloalkoxy, aryloxy, arylalkyloxy,  
 oxyheterocyclic, heterocyclic-substituted alkyloxy, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub>  
 5 cycloalkyl, thioaryl, thio-heterocyclic, arylalkylthio, heterocyclic-substituted  
 alkylthio, formyl, C<sub>1-7</sub> alkanoyl (acyl), carbamoyl, thiocarbamoyl, ureido,  
 thio-ureido, sulfonamido, hydroxylamino, alkoxyamino, mercaptoamino,  
 thioalkyl-amino, acylamino, thioacylamino, cyano, carboxylic acid or esters  
 or thioesters or halides or anhydrides or amides thereof, thiocarboxylic  
 10 acid or esters or thioesters or halides or anhydrides or amides thereof,  
 alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynyl-  
 amino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino,  
 heterocyclic amino, hydrazino, alkylhydrazino and phenylhydrazino;  
 optionally substituted heterocyclic radicals; aryl or heterocyclic radicals  
 15 substituted with an aliphatic spacer between the pteridine ring and said  
 aryl or heterocyclic radical, whereby said aliphatic spacer is a branched or  
 straight, saturated or unsaturated aliphatic chain of 1 to 4 carbon atoms  
 which may contain one or more functions, atoms or radicals independently  
 selected from the group consisting of carbonyl, thiocarbonyl, hydroxyl,  
 20 thiol, ether, thioether, acetal, thioacetal, amino, imino, oximino,  
 alkyloximino, amino-acid, cyano, acylamino, thioacylamino, carbamoyl,  
 thiocarbamoyl, ureido, thio-ureido, carboxylic acid or ester or thioester or  
 halide or anhydride or amide, thiocarboxylic acid or ester or thioester or  
 halide or anhydride or amide, nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl,  
 25 hydroxylamino, mercaptoamino, alkylamino, cycloalkylamino, alkenyl-  
 amino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino,  
 hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino,  
 alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl, sulfonamido and  
 halogen; branched or straight, saturated or unsaturated aliphatic chains of  
 30 1 to 7 carbon atoms optionally containing one or more functions, atoms or  
 radicals independently selected from the group consisting of halogen,

carbonyl, thiocarbonyl, hydroxyl, thiol, ether, thio-ether, acetal, thio-acetal, amino, imino, oximino, alkyloximino, aminoacid, cyano, acylamino, thioacylamino, carbamoyl, thiocarbamoyl, ureido, thio-ureido, carboxylic acid ester or halide or anhydride or amide, thiocarboxylic acid or ester or thioester or halide or anhydride or amide, nitro, thio C<sub>1-7</sub> alkyl, thio C<sub>3-10</sub> cycloalkyl, hydroxylamino, mercapto-amino, alkylamino, cycloalkylamino, alkenylamino, cycloalkenylamino, alkynylamino, arylamino, arylalkylamino, hydroxyalkylamino, mercaptoalkylamino, heterocyclic amino, hydrazino, alkylhydrazino, phenylhydrazino, sulfonyl, sulfinyl and sulfonamido; or R<sub>6</sub> together with R<sub>7</sub> and the carbon atoms in positions 6 and 7 of the pteridine ring forms a homocyclic or heterocyclic radical; and/or a pharmaceutically acceptable addition salt thereof and/or a stereoisomer thereof and/or a mono- or a di-*N*-oxide thereof and/or a solvate thereof and/or a dihydro- or tetrahydropteridine derivative thereof.

15

2. A pharmaceutical composition comprising as an active principle at least one pteridine derivative according to claim 1.

20

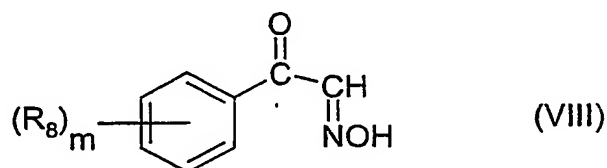
3. A pharmaceutical composition according to claim 2, further comprising one or more biologically-active drugs selected from the group consisting of immunosuppressant and/or immunomodulator drugs, antineoplastic drugs, and antiviral agents.

25

4. A pharmaceutical composition according to claim 2 or claim 3, further comprising one or more immunosuppressant drugs selected from the group consisting of cyclosporin A; substituted xanthines; pentoxifylline; daltroban, sirolimus, tacrolimus; rapamycin and derivatives thereof; leflunomide or an active metabolite or an analog thereof; mycophenolic acid and salts thereof; adrenocortical steroids; azathioprine, brequinar; gusperimus; 6-mercaptopurine; mizoribine; chloroquine; hydroxychloroquine; monoclonal antibodies with immunosuppressive properties; etanercept; infliximab; and kineret.

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5. A pharmaceutical composition according to claim 2 or claim 3, further comprising one or more immunomodulator drugs selected from the group consisting of acemannan, amiprilose, bucillamine, ditiocarb sodium, imiquimod, inosine pranobex, interferon- $\beta$ , interferon- $\gamma$ , lentinan, levamisole, pidotimod, romurtide, platonin, procodazole, propagermanium, thymomodulin, thymopentin and ubenimex.
6. A pharmaceutical composition according to claim 2 or claim 3, further comprising one or more antineoplastic drugs selected from the group consisting of alkaloids, alkylating agents, alkyl sulfonates, aziridines, ethylenimines, methylmelamines, nitrogen mustards, nitrosoureas, antibiotics, antimetabolites, folic acid analogs, purine analogs, pyrimidine analogs, enzymes, interferon and platinum complexes.
7. A pharmaceutical composition according to claim 2 or claim 3, further comprising one or more antiviral agents selected from the group consisting of retroviral enzyme inhibitors, HIV-1 IN inhibitors, nucleoside reverse transcriptase inhibitors, zidovudine, lamivudine, didanosine, stavudine, zalcitabine, non-nucleoside reverse transcriptase inhibitors, nevirapine, delavirdine, foscarnet sodium, HIV-1 protease inhibitors, saquinavir, ritonavir, indinavir, nelfinavir, acyclovir, cidofovir, cytarabine, edoxudine, famciclovir, floxuridine, ganciclovir, idoxuridine, penciclovir, sorivudine, trifluridine, valaciclovir, vidarabine, kethoxal, methisazone, moroxydine, podophyllotoxin, ribavirine, rimantadine, stallimycine, statolon, tromantadine and xenazoic acid.
8. A substituted phenylglyoxalmonoxime having the general formula (VIII):



wherein  $m$  is from 0 to 5, and wherein each substituent  $R_8$  is independently selected from the group consisting of halogen, cyano, piperidino, imidazol-1-yl, hydroxy, amino, protected amino, nitro, benzoxy, acetoxy,  $C_{1-7}$  alkoxy and  $C_{1-7}$  alkyl, said substituted phenylglyoxalmonoxime being an intermediate for making a pteridine derivative according to claim 1.

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ABSTRACT

5 This invention relates to a group of substituted pteridine derivatives, their  
pharmaceutically acceptable salts, N-oxides, solvates, dihydro- and tetrahydro-  
derivatives and enantiomers, possessing unexpectedly desirable pharmaceutical  
properties, in particular which are highly active immunosuppressive agents, and  
as such are useful in the treatment in transplant rejection and/or in the treatment  
of certain inflammatory diseases. These derivatives are also useful in preventing  
or treating cardiovascular disorders, allergic conditions, disorders of the central  
10 nervous system, TNF- $\alpha$  related disorders and cell proliferative disorders.

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Figure 1

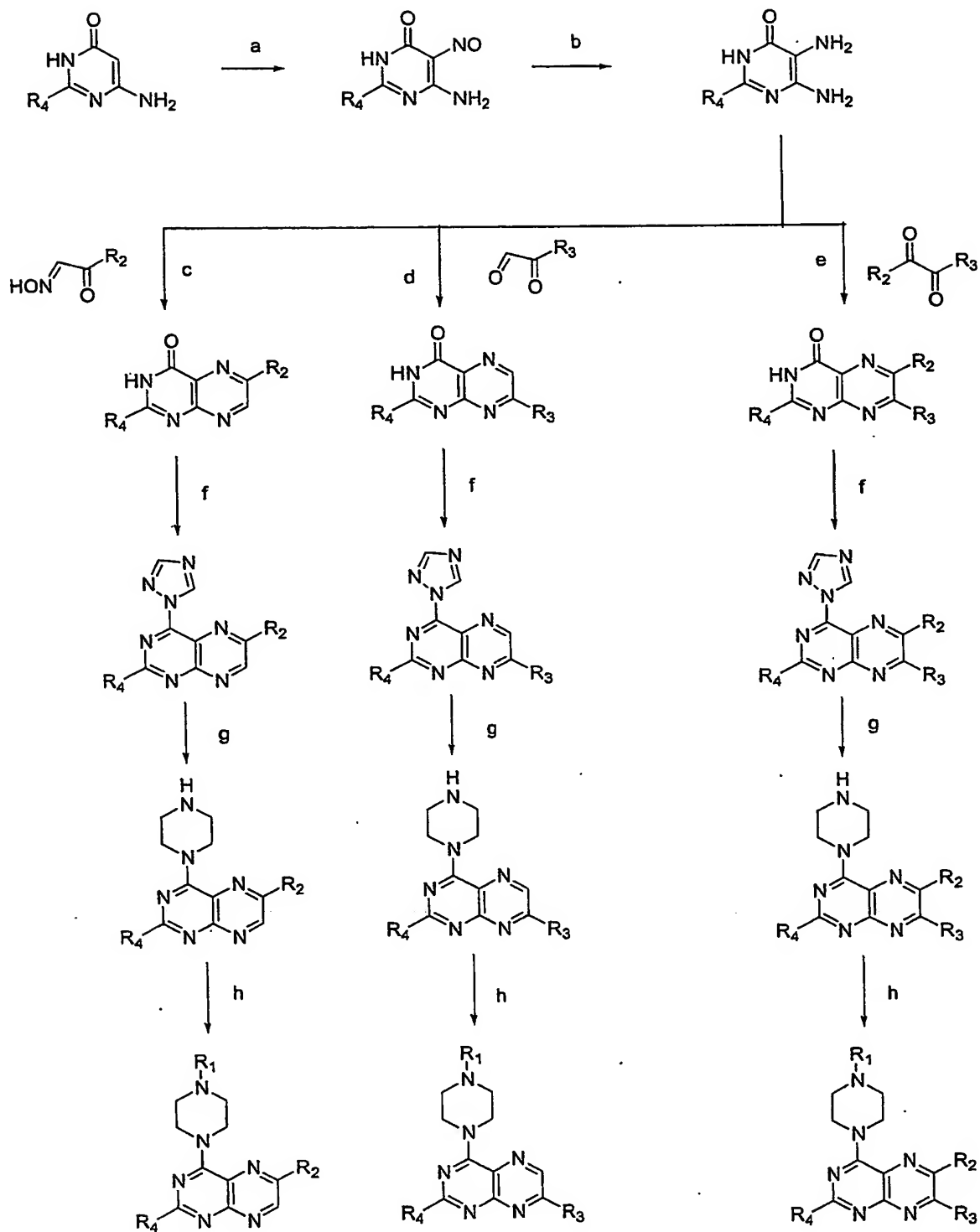


Figure 2

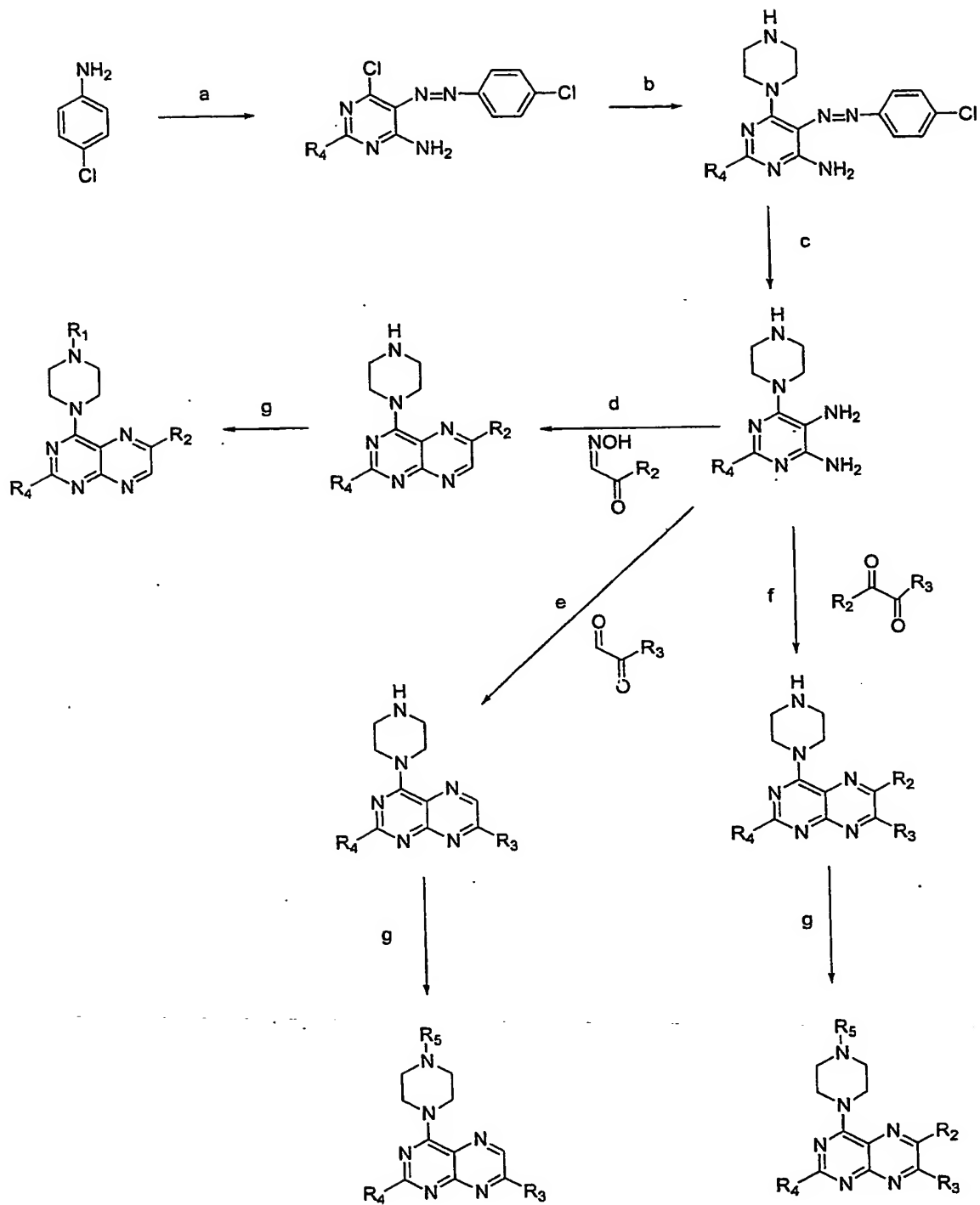


Figure 3

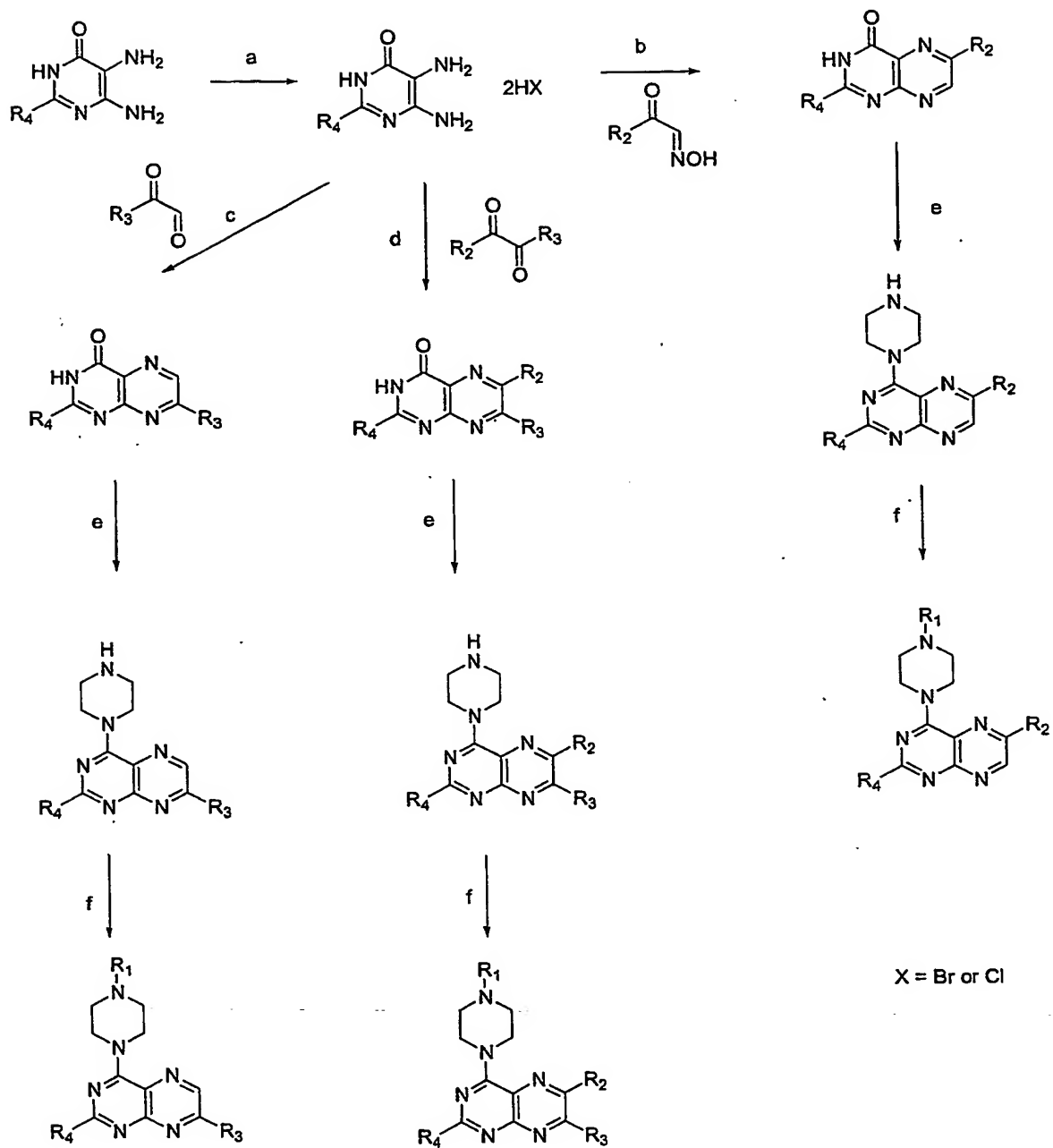


Figure 4

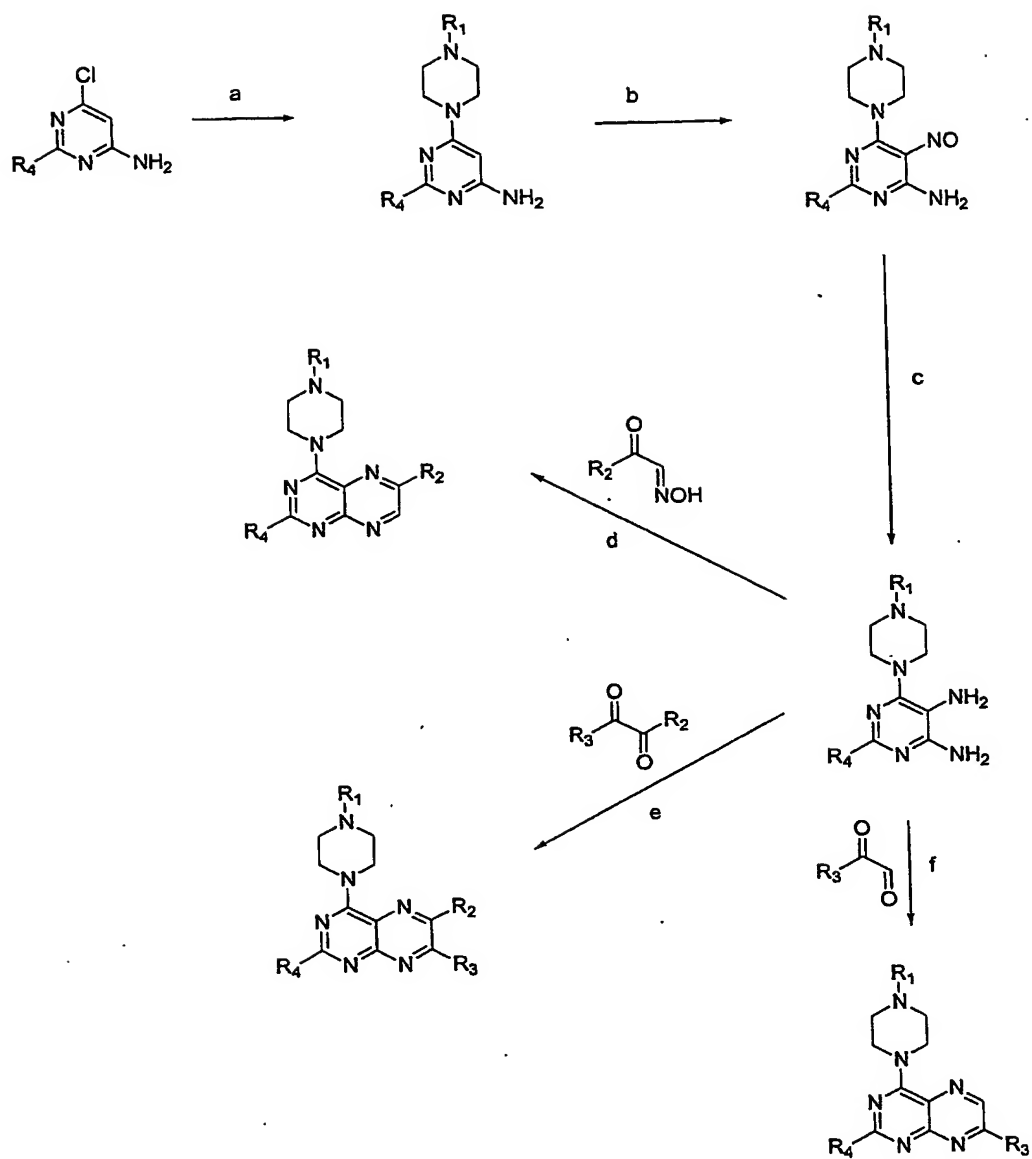


Figure 5

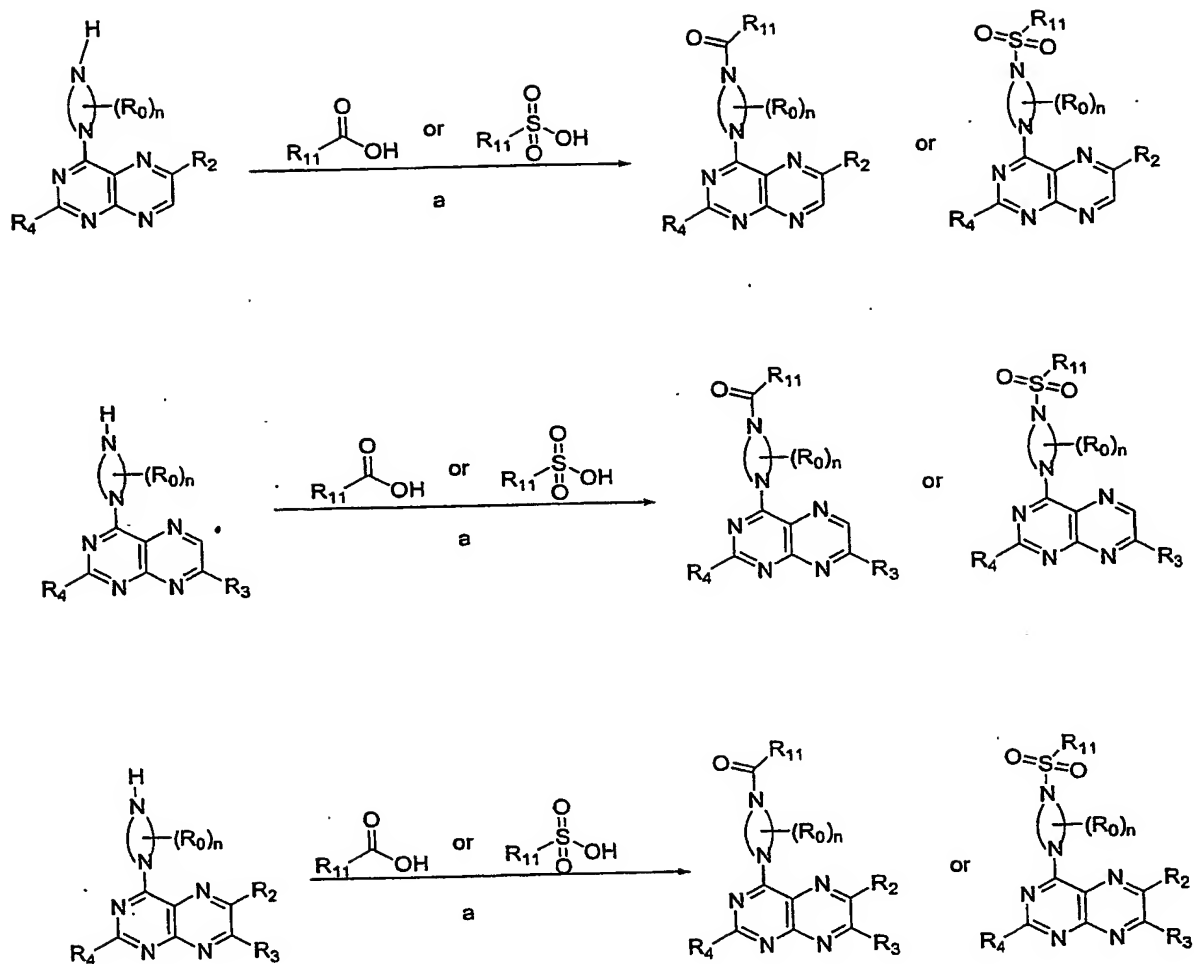


Figure 6

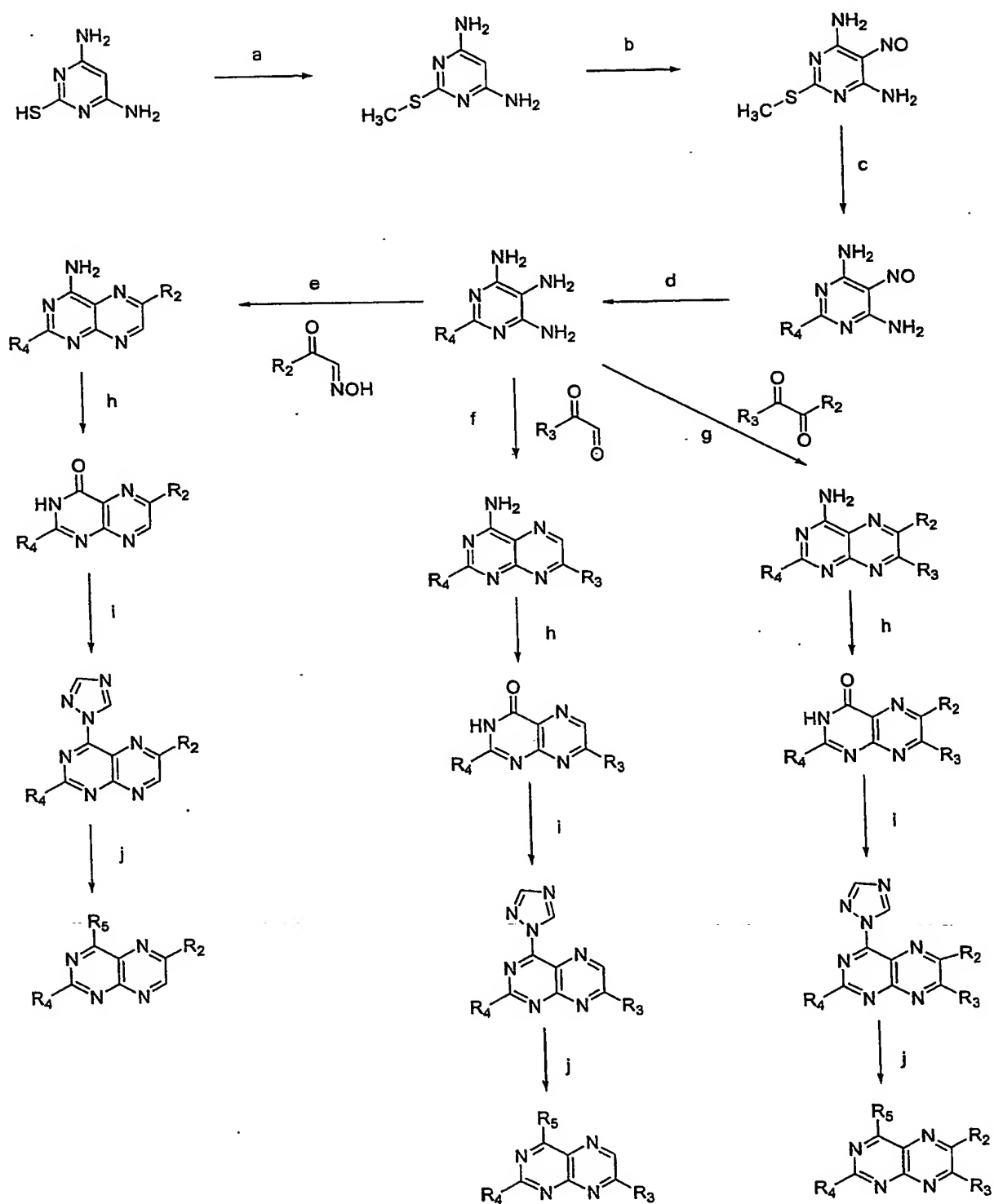


Figure 7

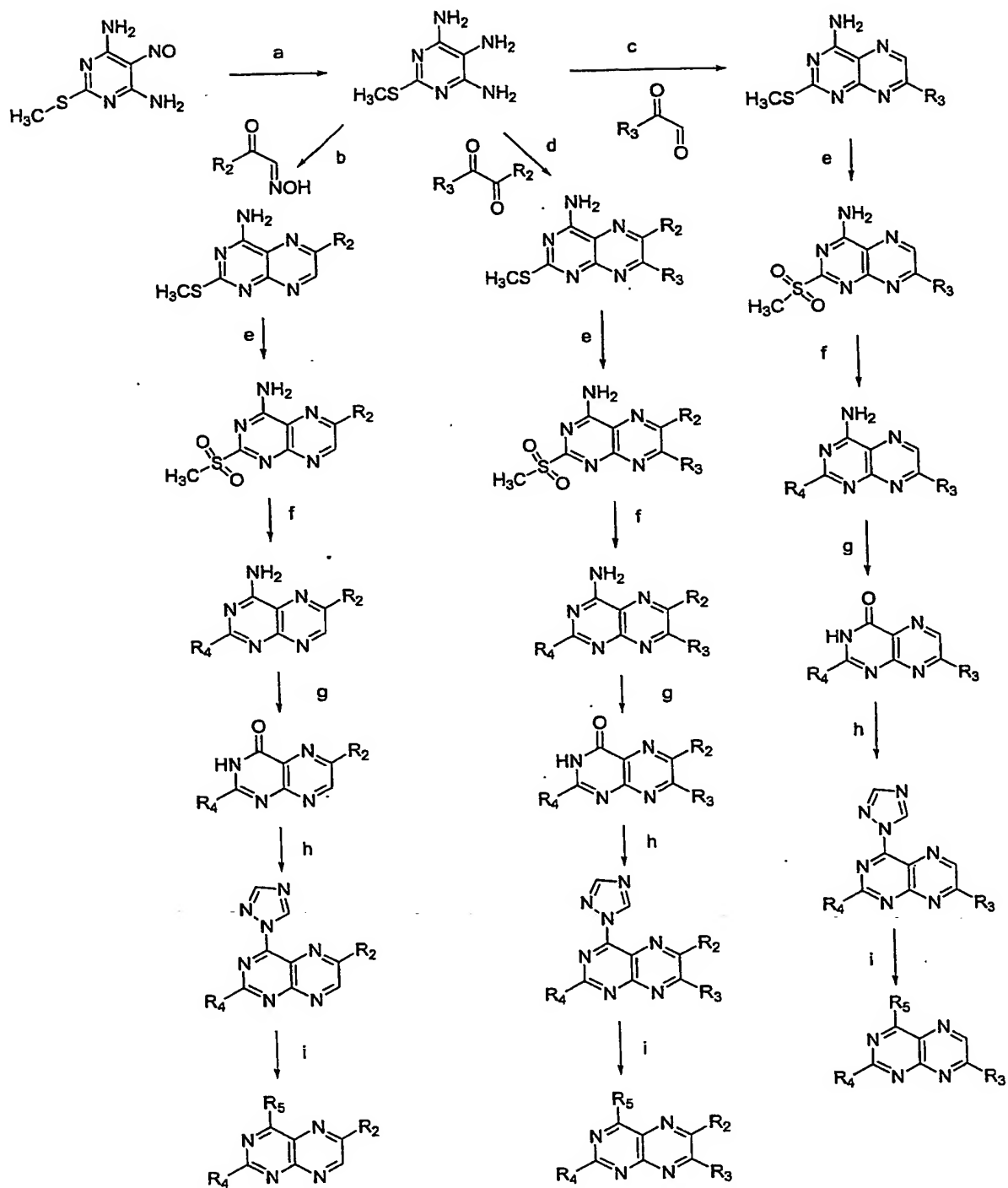




Figure 8

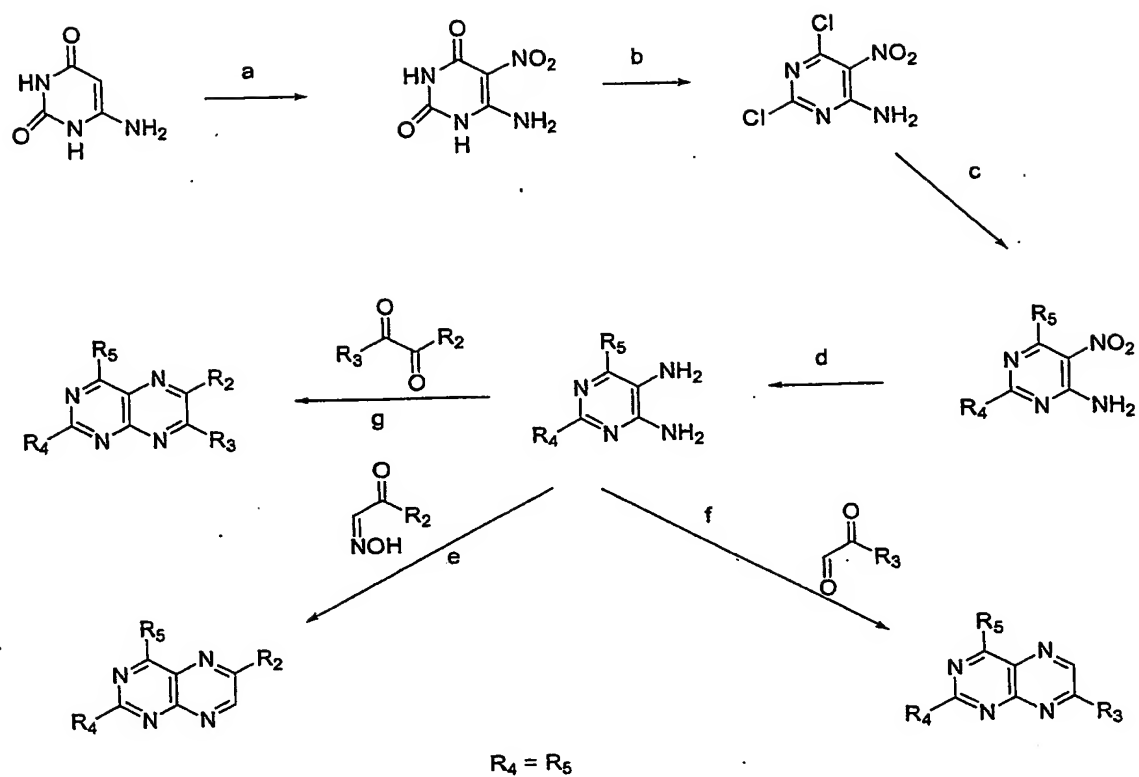


Figure 9

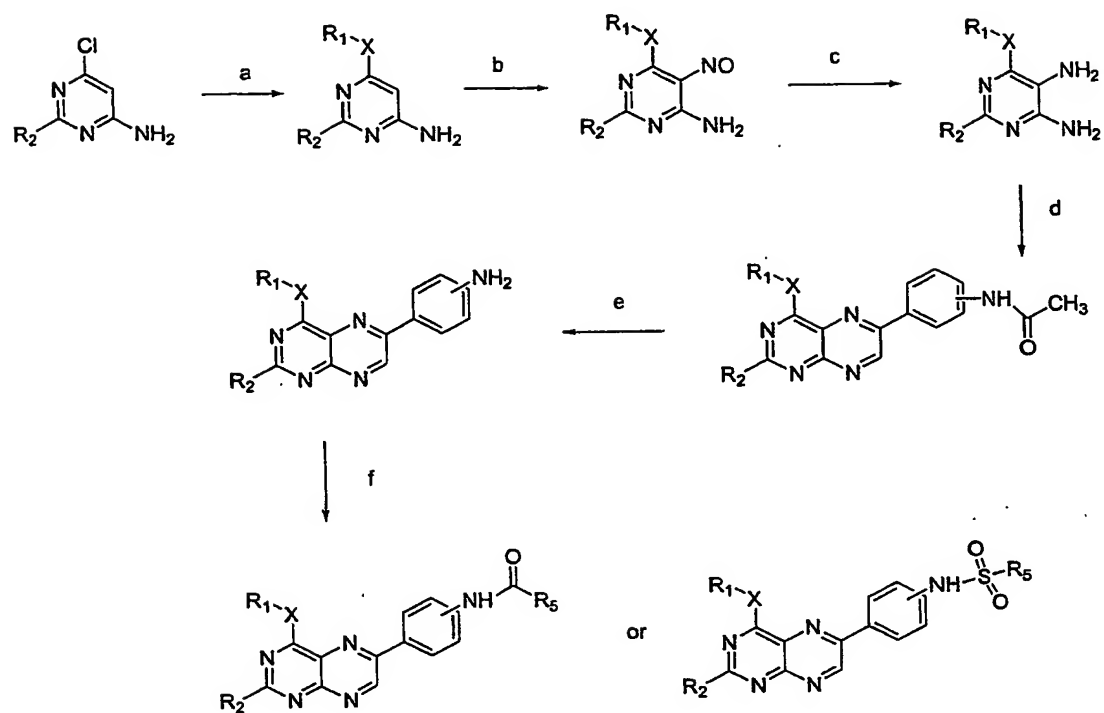


Figure 10

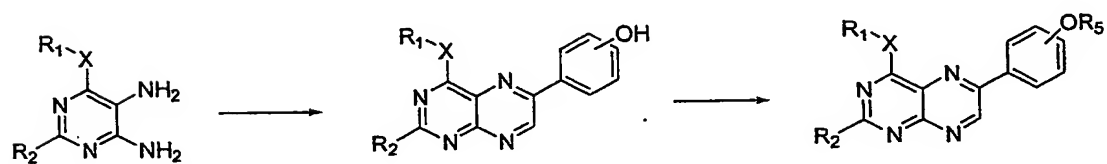


Figure 11

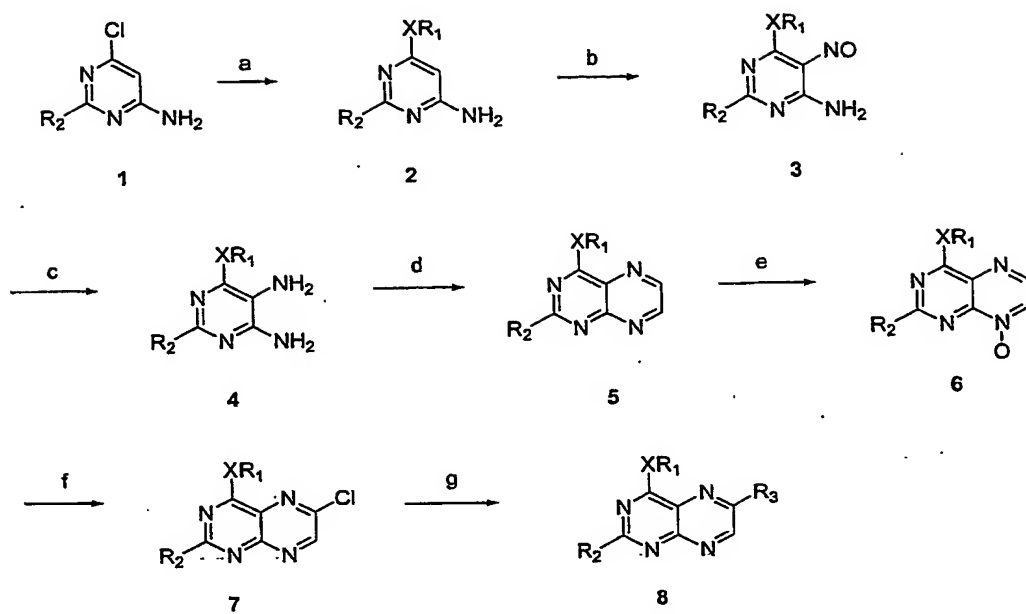


Figure 12

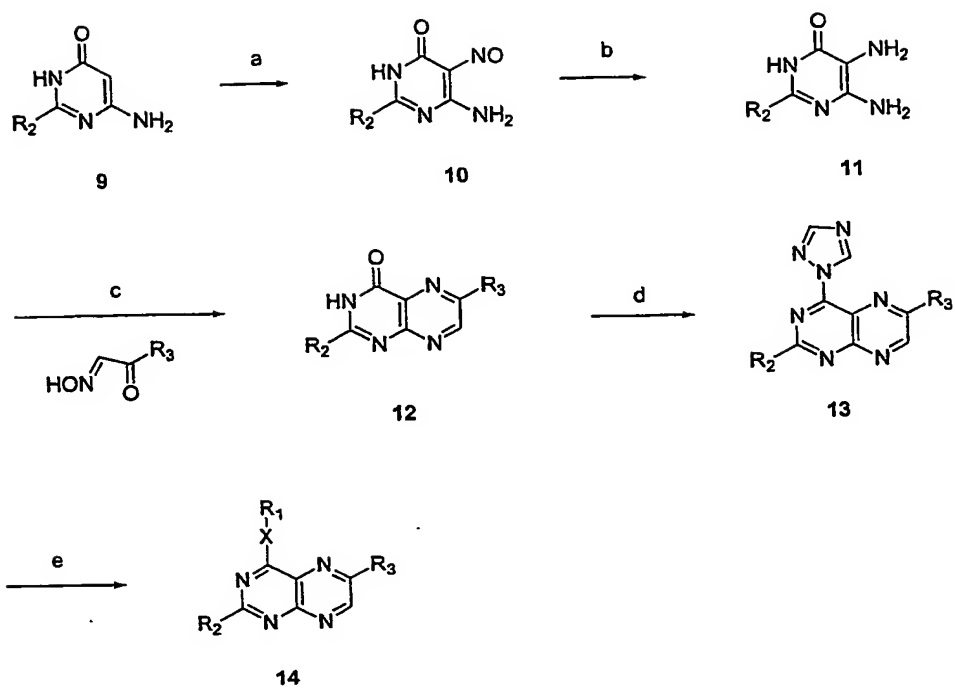
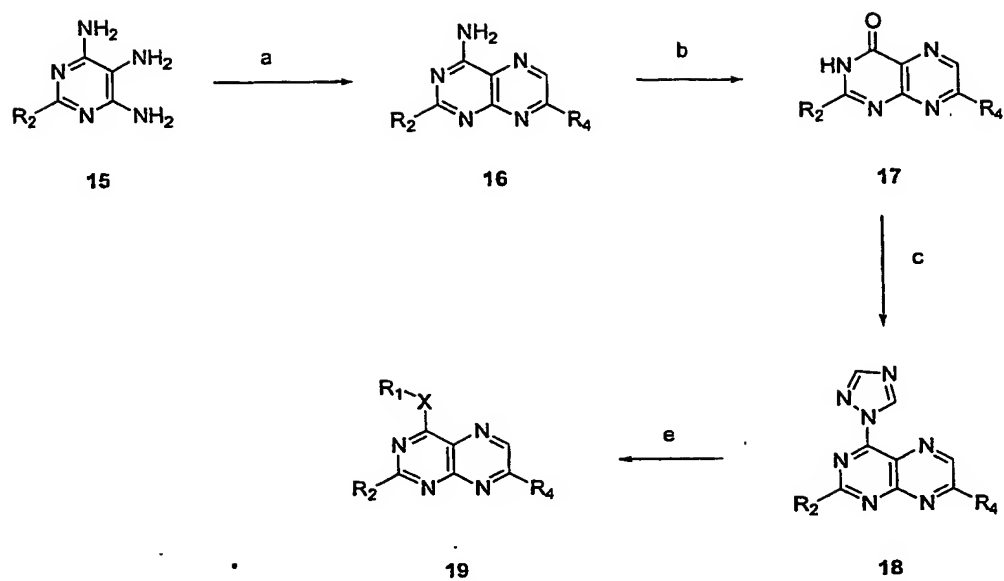


Figure 13



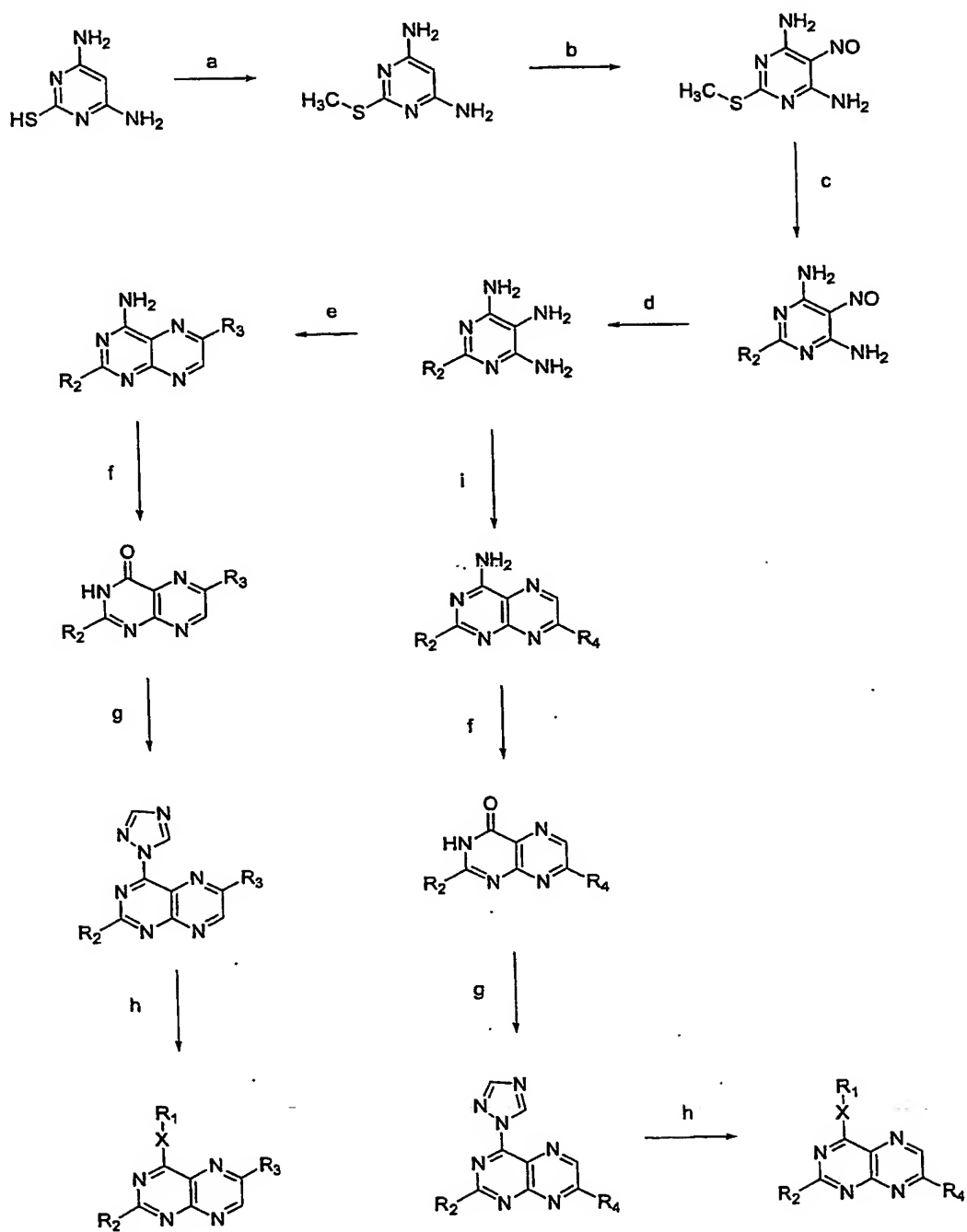
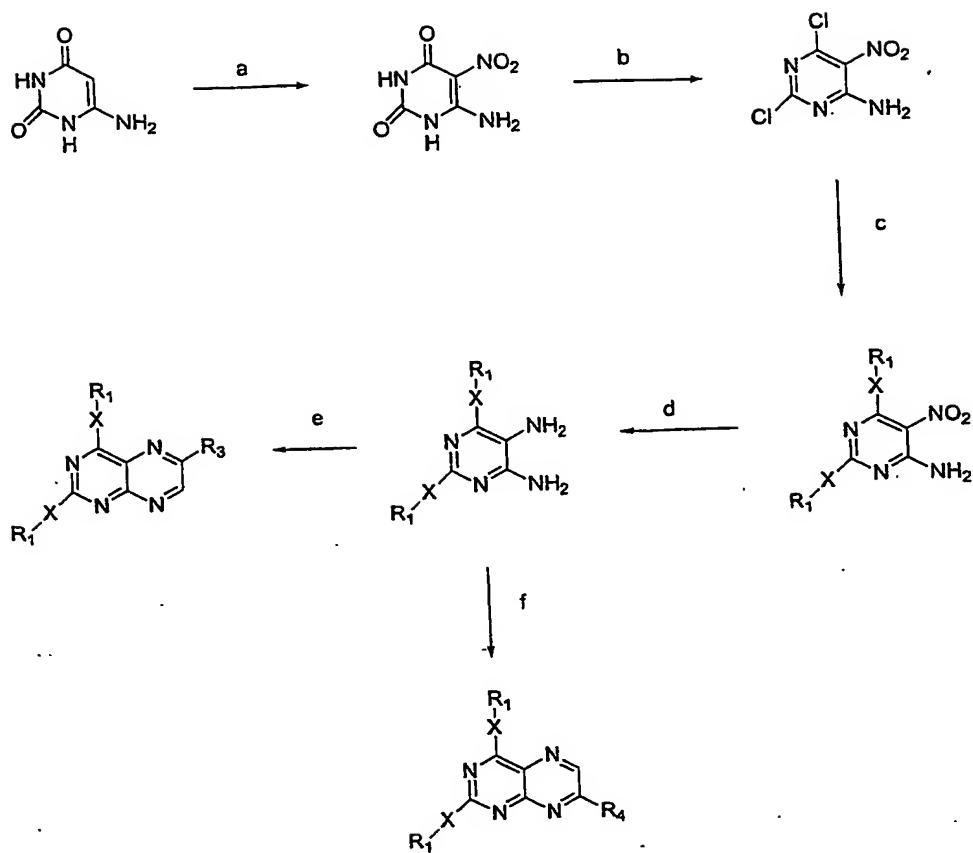


Figure 15



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